DOI 10.2478/helm-2019-0013

HELMINTHOLOGIA, 56, 2 • 2019

EDITORIAL

Laudatio to Professor Pavol Dubinský on his 80th Anniversary

February 12, 2019 was the 80th birthday of Prof. D.V.M. Pavol Dubinský, D.Sc., one of the most noteworthy Slovak parasitologists and the former director of the Institute of Parasitology, Slovak Academy of Sciences, in Košice (IP SAS).

Prof. Dubinský graduated from School of Agriculture (now University of Veterinary Medicine and Pharmacy) in Košice in 1962, and started his meticulous scientific career at the IP SAS (formerly Helminthological Institute). At the Department of Morphology and Physiology he dealt with the effect of ultraviolet radiation on the survival of helminth propagative stages and physiology of helminth developmental stages, as well as their adaptation to the parasitic mode of existence, and its impact on host organisms.

In 1988 he became the Director of IP SAS and held this position for five terms until 2007. After the establishment of the independent Slovak Republic in 1993, he ensured the formation of independent and complex parasitological research. His attention focused on recent global problems in parasitology related to the existing trends in the spread of zoonotic and vector-borne diseases. He was among the key researchers in the projects of the European scientific programs (FP5) EchinoRisk and TrichiPorse focused on alveolar echinococcosis and trichinellosis.

Prof. Dubinský was one of the founding members of the Slovak Society for Parasitology and served as its president for the period of 14 years (1994 to 2008). As an extensively recognized expert, he was a member of several international organizations. He worked as Vice President of the World Federation of Parasitologists, Slovak representative in the International Commission on Trichinellosis and member of the World Association for the Advancement of Veterinary Parasitology. For many years he had been editor-in-chief of the internationally recognized scientific journal Helminthologia. He worked as editorial board member in several scientific and professional journals.

Among many awards for the scientific merit and popularization of science in Slovakia, prof. Dubinský was in 2008 awarded by the *2nd Class Pribina Cross*. This medal was presented by the President of the Slovak Republic "for his significant contribution to social and economic growth of the Slovak Republic".

Prof. Dubinský also implemented the results of parasitological research into the veterinary practice by participation in extensive lecturing activities. He supervised large numbers of diploma and doctoral students and worked in pertinent committees for habilitation and inauguration of university pedagogical staff.

In the present, Prof. Dubinský is the designated Emeritus Scientist at his home institute IP SAS in Košice. He is still active as an scientist, management consultant, and a mentor for young scientists and students. In the leisure time he devotes himself to his two great hobbies - beekeeping and gardening.

All former fellow workers want to express to Prof. Dubinský their heartfelt appreciation for all professional opportunities he had provided for many years and for his friendly and caring attitude. They pray for his solid health, wish a lot of creative powers, happiness and joy in his personal life!

Martina Miterpáková

DOI 10.2478/helm-2019-0006

HELMINTHOLOGIA, 56, 2: 87 – 92, 2019

Preliminary evidence for the occurrence of β-tubulin isotype 3 polymorphisms in *Fasciola hepatica* isolates in cattle and sheep in Turkey

S. SADET CANAKOGLU[1], S. SİMSEK[2*], I. BALKAYA[3], S. GUNYAKTİ KİLİNC[2]

[1]Department of Animal Sciences, Directorate of Provincial Food Agriculture and Livestock, Kayseri, Turkey;
[2*]Department of Parasitology, Faculty of Veterinary Medicine, University of Firat, 23119, Elazig, Turkey, E-mail: *ssimsek@firat.edu.tr*;
[3]Department of Parasitology, Faculty of Veterinary Medicine, University of Ataturk, Erzurum, Turkey

Article info

Received August 8, 2018
Accepted January 24, 2019

Summary

Fasciolosis caused by *Fasciola hepatica* is a common parasitic disease of livestock especially sheep and cattle. In this study molecular characterization of β-tubulin isotype 3 gene in *Fasciola hepatica* isolates from cattle and sheep in Turkey was carried out. For this purpose a total of 80 adult *Fasciola hepatica* isolates were collected from 20 sheep and 20 cattle in Kayseri and Erzurum provinces. PCR-RFLP was performed on β-tubulin isotype 3 gene and *MboII* revealed two fragments of approximately 350 bp and 390 bp, whereas *HphI* enzyme yielded 210, 340 and 540 bp bands, *HindII* yielded 380 and 450 bp bands in all samples. A total of 80 isolates were tested by SSCP and all of them presented the same band profiles. Six samples (4 sheep and 2 cattle) were randomly selected and DNA sequence of a 935 bp coding fragment of β-tubulin isotype 3 was performed. Sheep samples were more polymorphic than the cattle. This β-tubulin isotype 3 gene polymorphism of *F.hepatica* isolates from sheep and cattle of two distinct geographical areas of Turkey have been investigated for the first time.

Keywords: *Fasciola hepatica*; β-tubulin 3; Polymorphism; Sheep; Cattle; Turkey

Introduction

The liver fluke, *Fasciola hepatica,* is a worldwide distributed trematode parasite of great veterinary importance, which causes significant economic losses (feed conversion efficiency, poor carcass conformation, malnutrition, decreased fertility and milk yield) in livestock (Khoramian *et al.*, 2014). It is a food-borne trematode zoonosis and is classified as neglected tropical disease (WHO, 2015). Fasciolosis is an asymptomatic infection in cattle and causes more serious pathological implications in sheep. Acute fasciolosis in sheep occurs seasonally and manifests itself with a relatively swollen and painful abdomen; and sudden death occurs within 2 – 6 weeks after infection. Advanced chronic infection is fatal in sheep, this rate is less in cattle (Mas-Coma *et al.*, 2005). Survival is longer in cases of significant liver damage (7 – 10 weeks) in subacute disease, however, death can occur due to hemorrhage and anemia. Although chronic fasciolosis can be seen during all seasons of the year, it occurs primarily in autumn and winter. Anemia, unthriftiness, submandibular edema and decreased milk production are the most important symptoms. The benzimidazole derivative triclabendazole is a highly specific drug for the treatment of fasciolosis (Fairweather & Boray, 1999). Microtubules are highly conserved among species, the polymers of α and β-tubulin, in heterodimer form. The *Fasciola hepatica* genome codes for at least five α and six β-tubulin isotypes (Ryan *et al.*, 2008). β-tubulin proteins have been characterised from a variety of helminth species. To date, however, there is no data about the polymorphism in the β-tubulin gene from isolates in Turkey.

In the present study we investigated the possible polymorphism in the 935 bp fragment of β-tubulin isotype 3 gene of *F. hepatica*

* – corresponding author

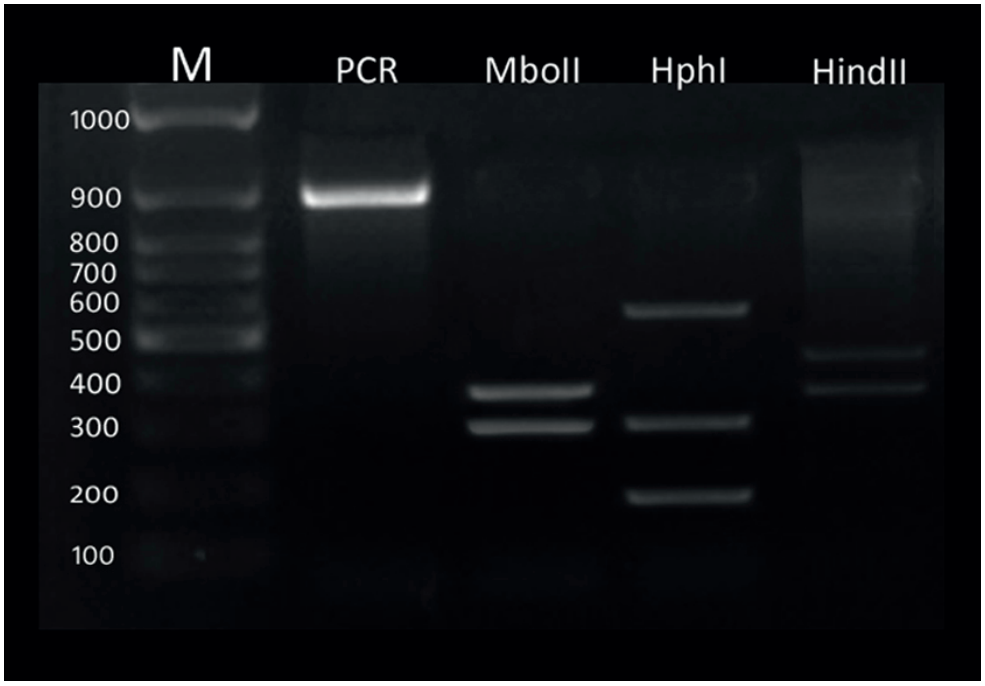

Fig. 1. PCR and PCR-RFLP results of *Fasciola hepatica* β-tubulin gene. M: Marker (100 bp); PCR: β-tubulin gene (935 bp); MboII: Restriction with *MboII* (350 and 390 bp); HphI: Restriction with *HphI* (210, 340 and 540 bp); HindI: Restriction with *HindI* (380 and 450 bp).

which has been collected from sheep and cattle isolates from different geographical areas of Turkey.

Materials and Methods

Sample Collection

The study defines an isolate as a single adult *F. hepatica* from each separate liver. A total of 80 isolates from livestock (sheep n=20, cattle n=20) originating from Erzurum and Kayseri provinces were collected over a period of 2 years (January 2014-December 2015) during random visits to licensed slaughterhouses in Turkey. The isolates were preserved in 70 % ethanol and were transferred to the laboratory for genomic DNA isolation.

Genomic DNA Isolation

Total genomic DNA (gDNA) was isolated from the adult parasite tissue using the GeneAll® Tissue Kit (Korea) according to the manufacturer's instructions. Prior to gDNA isolation, the anterior part of each adult parasite was dissected and ethanol residue was removed by at least five times repeated wash in 600 µl 1 M PBS (pH=7,4). gDNA quality was evaluated by optical density ratio (260/280 nm), using a NanoDrop 8000 Spectrophotometer (Thermo Scientific). gDNA isolated samples were used for further PCR and sequence analysis.

Polymerase Chain Reaction (PCR)

A fragment (935 bp) of β-tubulin isotype 3 gene was amplified using the primers β-tub295f (5'-AAYAAYTGGGCYAARGGNCAYTA-3') and β-tub1230r (5'-TCRGTRAAYTCCATYTCRTCCAT-3') previously reported (Ryan *et al.,* 2008). We used the following conditions: initial denaturation step of 10 min at 95 °C and 40 cycles of 1 min at 94 °C (denaturation), 1 min at 56 °C (primer annealing), 1 min at 72 °C (extension) followed by a final extension step of 10 min at 72 °C. Amplicons were electrophoretically visualized on an ethidium bromide stained 1.4 % agarose gel under UV.

Restriction Fragment Lenght Polymorphism (PCR-RFLP)

The restriction enzymes used for this procedure were determined using previously published sequences (HM535826, HM535800 and HM535806) in PubMed. NEBCutter V2.0 program was used to determine which enzymes cut the sequence and *MboII* (5 U/µl, 300U Thermo Scientific), *HphI* (5 U/µl, 300U Thermo Scientific) and *HindII* (5 U/µl, 300U Thermo Scientific) were used in this

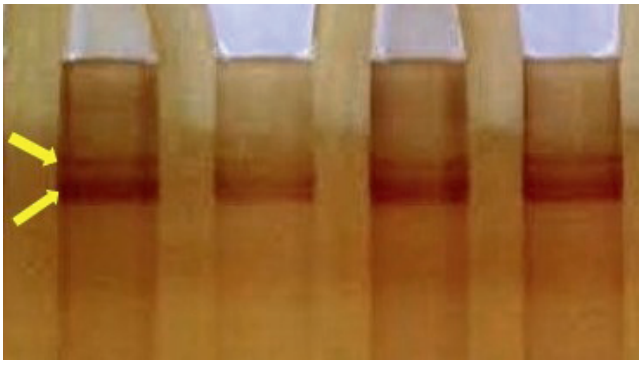

Fig. 2. SSCP band profiles of some selected samples.

study. PCR products were digested during 3 hours with restriction enzymes at 37 °C using buffers recommended by the manufacturer (Thermo Scientific) in a final 18,7 µl volume reaction mix, containing 10 µl of PCR product, 1 µl from each enzyme (*MboII*, *HphI* and *HindII*), 2 µl restriction buffer, 0.2 µl BSA and 5.5 µl distilled water. The restriction fragments were separated on 3 % agarose gel, stained with ethidium bromide and photographed (Vilber Lourmat Quantum-ST5-1100/26MX).

Single Stranded Conformation Polymorphism (SSCP)

The method was performed according to Simsek *et al.* (2012). Eight microliters of each PCR product were mixed with 12 µl of

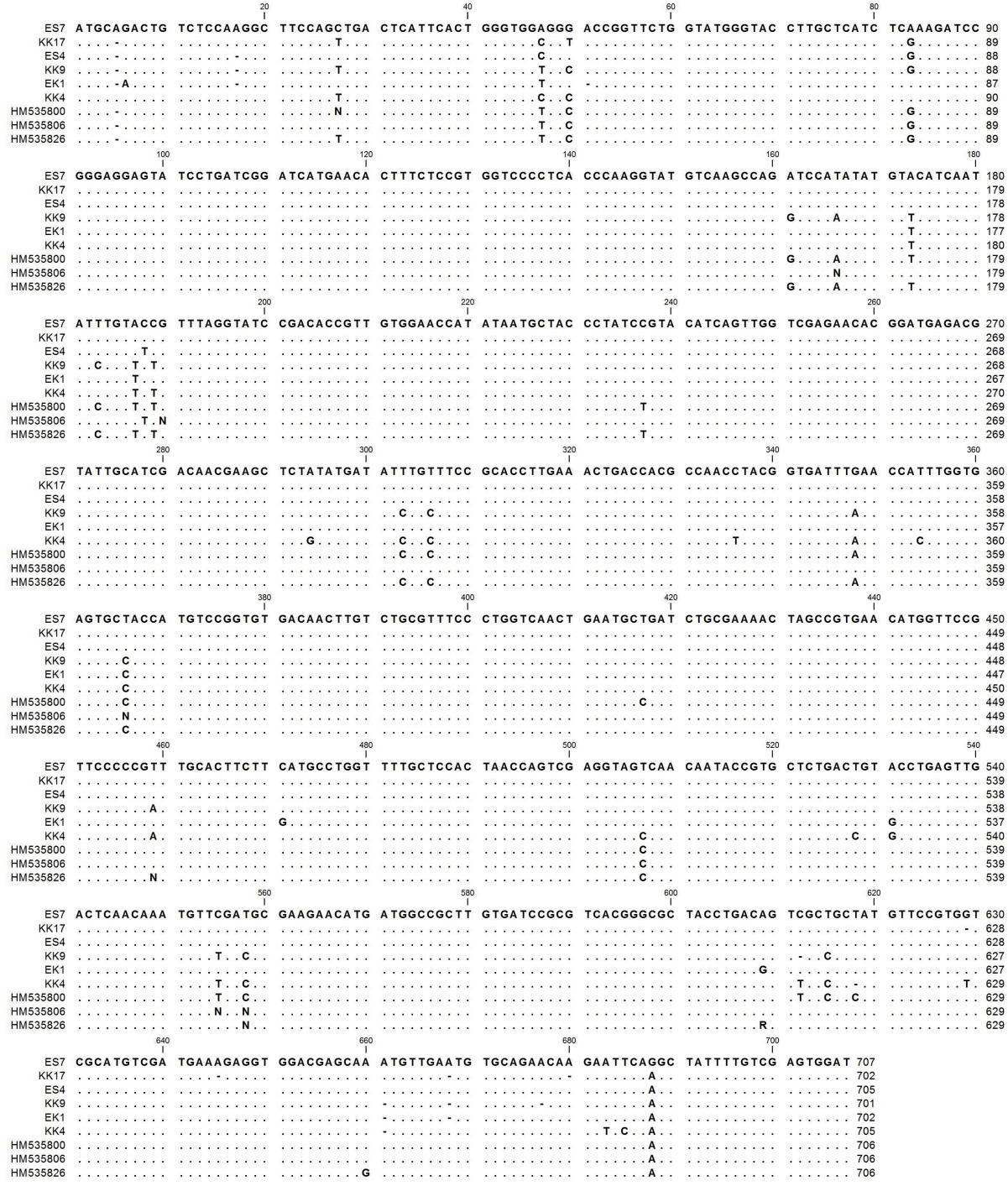

Fig. 3. Nucleotide sequence alignment of samples analyzed in this study using with the reference sequences (HM535800, HM535806 and HM535826).

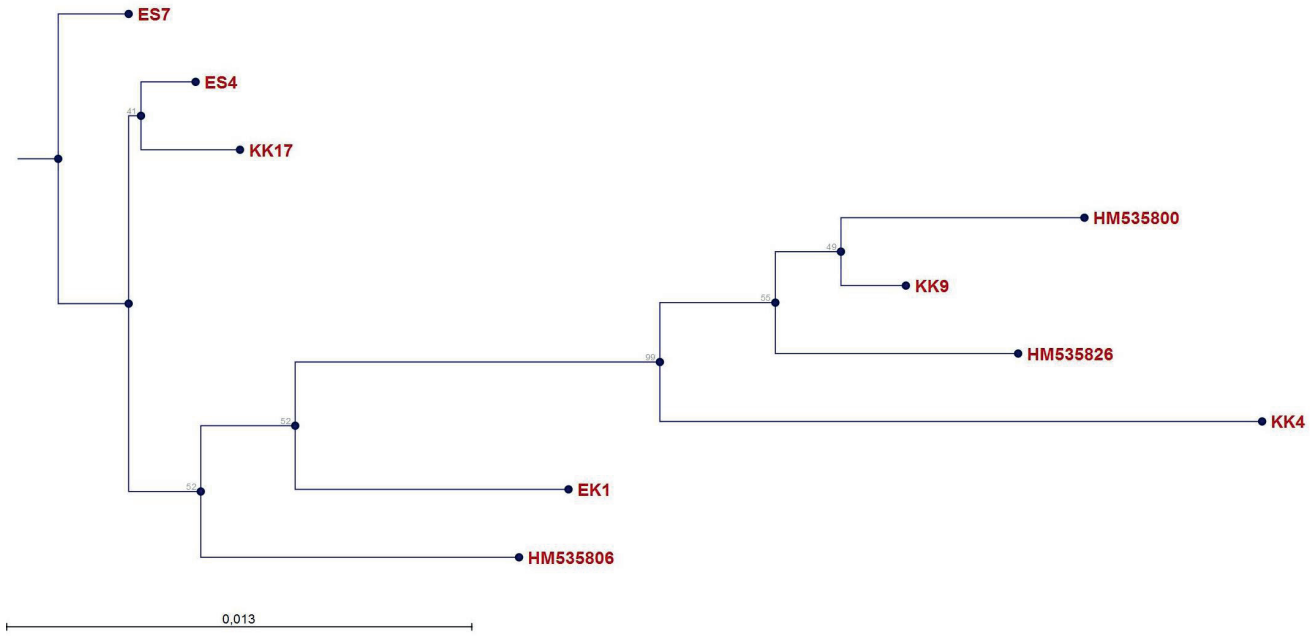

Fig. 4. Phylogenetic tree view of the aligned nucleotide sequences using with the reference sequences (HM535800, HM535806 and HM535826).

denaturing buffer (10 mM NaOH, 95 % formamide, 0.05 % bromophenol blue and 0.05 % xylene cyanole). Following the denaturation at 95 °C for 10 min and subsequent snap-cooling on a freeze block (-20 °C), 10 µl of individual samples were loaded into the gel. The electrophoresis was carried out in 12 % acrylamide-bisacrylamide (49:1) non-denaturing gels containing 10 % glycerol, at 200 V constant current during 3 h at room temperature. The band profiles were displayed by silver staining.

DNA Sequence Analysis

The *F. hepatica* partial β-tubulin gene sequences were obtained automatically. Nucleotide sequence analysis was undertaken by BLAST algorithms and databases from the NCBI (http://www.ncbi.nlm.nih.gov). The following accession numbers were given to sequences: KU523368, KU523369, KU523370, KU523371, KU523372 and KU523373.

Ethical Approval and/or Informed Consent

For this study formal consent is not required (Firat University, Local Ethics Committee of Animal Experiments, Decision no: 2014/73).

Results

A 935 bp fragment of β-tubulin isotype 3 gene was amplified through PCR in 80 *F. hepatica* isolates (Fig. 1). The molecular characterization of *F.hepatica* was carried out on the basis of PCR-RFLP and SSCP analysis of β-tubulin isotype 3 gene and sequence analysis as well. Three restriction enzymes (*MboII*, *HphI* and *HindII*) were used to differentiate the β-tubulin isotype 3 gene of adult flukes. Different band patterns were observed in PCR-RFLP after enzyme digestion. The PCR-RFLP profile obtained from *MboII* revealed two fragments of approximately 350 bp and 390 bp (Fig. 1), whereas *HphI* enzyme yielded 210, 340 and 540 bp bands (Fig. 1), and *HindII* yielded 380 and 450 bp bands (Fig. 1) in all samples. A total of 80 isolates were tested by SSCP and all of them presented the same band profile (Fig. 2). Six samples (n=4 from sheep in Kayseri, n=2 from cattle in Erzurum) were randomly selected and sequenced. Four of those samples were isolated from sheep in Kayseri (KK4, Accession no: KU523371; KK9, Accession no: KU523372; KK17, Accession no: KU523373) and Erzurum (EK1, Accession no:KU523368) while the remaining two samples were isolated from cattle in Erzurum (ES4, Accession no: KU523369 and ES7 Accession no: KU523370). Multiple sequence alignment was performed including the previously published sequences (HM535800, HM535806 and HM535826) used for alignment and the results are shown in Fig. 3. According to alignment results, much more polymorphic sites were seen in sheep isolates (KK4, KK9 ve KK17) than the cattle. It has been illustrated in a genetic tree as well (Fig. 4). Amino acid comparisons of the samples were performed and mutations at different ratios at the sequence were observed (Fig. 5). The β-tubulin 3 gene sequences described in the current study were compared to those described previously (HM535800, HM535806 and HM535826). Thyrosin amino acid was detected in all sequenced and control sequences in 200th position.

Discussion

Molecular studies on the genetic diversity of *Fasciola* species using

Fig. 5. Alignment of the partial β-tubulin isotype 3 amino acid sequence with other relevant sequences (HM535800, HM535806 and HM535826). Alignment has been started from 99. position of the reference sequences. Amino acid residues that are conserved in at least 2 proteins are indicated in white back ground. Rectangle on the alignment indicate Tyr amino acid in the 200th position.

mt-DNA have confirmed genetically varied populations (Elliot *et al.,* 2014). Level of genetic diversity of *F. hepatica* suggests that it may have the potential to adapt to selection pressure within the environment with clonal reproduction in snails allowing rapid dispersal of resistant populations (Prugnolle *et al.,* 2005). Microtubules are the major promoters of cytoskeleton and are widespread in all eukaryotic cells. They accompany some functions including cell division and intracellular transport mechanisms (Nogales *et al.*, 1998). It has been previously established that the adult flukes express at least six tubulin isotypes designated as *F. hepatica* β-tub1 to β-tub6 (Ryan *et al.*, 2008). The gene coding β-tub isotype 3 was chosen in the current study. The total length of the gene is 1335 bp. Selected primers were used for amplification of region 935 bp in length. This is a region encoding 242 amino acids of the protein sequence.

Cwiklinski et al. (2015) highlighted tubulins exhibit the highest log fold-changes in expression throughout *F. hepatica* development. They identified the full complement of five α-tubulin and six β-tubulin isotypes and found duplication of β-tubulin isotype 3. We investigated levels of polymorphism among individual *F. hepatica* isolates collected from sheep and cattle by partial sequencing of the β-tubulin isotype 3. It has been detected more nucleotide polymorphism in sheep isolates than cattle. This may be due to the more common prevalence of sheep fasciolosis than the cattle in Turkey.

In our study, DNA sequences of 6 randomly selected samples were obtained from samples amplified by β-tubulin isotype 3 gene PCR of 80 *F. hepatica* isolates from Erzurum and Kayseri provinces. Sequence analysis resulted in amino acid comparisons of the samples, we observed the Thyrosin (Y) in the 200th position in all sequenced samples. Ryan *et al.* (2008) also identified Tyrosine at position 200 of the β-tubulin isotypes 1 – 3, Phenylalanine (F) in isotype 4 and 6 in two samples and Leucine in isotype 5 in one.

Teofanova *et al.* (2011) examined mutation and polymorphism in the β-tubulin 3 gene in 204 *F. hepatica* isolates obtained from Greece (n = 143), Poland (n = 48) and Bulgaria (n = 13) using sequence analysis. As a result, two major lineages for the 28S rDNA gene based on the highly polymorphic 105th nucleotide position

have been found. The basic lineages were observed within Greek, Bulgarian, and Polish *F. hepatica* populations but the distribution of additional haplotypes differed between the populations from the three countries. For the β-tubulin isotype 3 gene multiple polymorphic sites were revealed but no explicit clades. The SNPs were spread unequally in all studied geographical regions with an evident distinction between the Greek and Polish specimens. In our study, a few polymorphic points were identified in the sequenced sheep and cattle samples. These results indicate that phylogenetic analysis based on partial sequences of β-tubulin isotype 3 belonging to *F. hepatica* may support the polymorphism and allow understanding of the variation on the β-tubulin gene. Our findings suggest that the genetic diversity of *F. hepatica* β-tubulin isotype 3 of sheep and cattle isolates still continues. However for the better understanding of the sequence variability it needs to more lengths partial sequences.

In conclusion, this is the first report of a β-tubulin isotype 3 characterization in Turkey. The results of the current study contributed to understanding of the genetic structure and heterogeneity of *F. hepatica* β-tubulin isotype 3 in Turkey. However it needs further studies by using more sequenced samples.

Conflict of Interest

The authors fully declare that there is no financial or other potential conflict of interest.

Acknowledgements

This study has been supported by a grant from Firat University Scientific Research Projects Unit (Project No: VF.14.05). We also would like to thank to Dr. Alan Trudgett for the kind helps. The authors thank to Naunain Mehmood for the language edition.

References

Cwiklinski, K., Dalton, J.P., Dufresne, P.J., La Course, J., Williams D.J., Hodgkinson, J., Paterson, S. (2015): The *Fasciola hepatica* genome: gene duplication and polymorphism reveals adaptation to the host environment and the capacity for rapid evolution. *Genome Biol.,* 3(16):71. doi: 10.1186/s13059-015-0632-2

Elliott, T., Muller, A., Brockwell, Y., Murphy, N., Grillo, V., Toet, H.M., Anderson, G., Sangster, N., Spithill, T.W. (2014): Evidence for high genetic diversity of NAD1 and COX1 mitochondrial haplotypes among triclabendazole resistant and susceptible populations and field isolates of *Fasciola hepatica* (liver fluke) in Australia. *Vet. Parasitol.,* 200(1-2): 90-6. DOI: 10.1016/j.vetpar.2013.11.019

Fairweather, I., Boray, J.C. (1999): Fasciolicides: efficacy, actions, resistance and its management. *Vet. J.,* 158: 81 – 112

Khoramian, H., Arbabi, M., Osqoi, M.M., Delavari, M., Hooshyar, H., Asgari, M. (2014): Prevalence of ruminants fascioliasis and their economic effects in Kashan, center of Iran. *Asian Pacific J. Trop. Biomed.,* 4: 918 – 922. DOI: 10.12980/APJTB.4.2014APJTB-2014-0157

Mas-Coma, S., Bargues, M.D., Valero, M.A. (2005): Fascioliasis and other plant-borne trematode zoonoses. *Int. J. Parasitol.,* 35: 1255 – 78. DOI: 10.1016/j.ijpara.2005.07.010

Nogales, E., Wolf, S.G., Downing, K.H. (1998): Structure of the alpha beta tubulin dimer by electron crystallography. *Nature,* 391: 199 – 203

Prugnolle, F., Liu, H., De Meeus, T., Balloux, F. (2005): Population genetics of complex life-cycle parasites: an illustration with trematodes. *Int. J. Parasitol.* 35: 255 – 263

Ryan, L.A., Hoey, E., Trudgett, A., Fairweather, I., Fuchs, M., Robinson, M.W., Chambers, E., Timson, D., Ryan, E., Fetwell, T., Ivens, A., Bentley, G., Johnston, D. (2008): *Fasciola hepatica* expresses multiple a- and b-tubulin isotypes. *Mol. Biochem. Parasitol.,* 159: 73 – 78. DOI: 10.1016/j.molbiopara.2008.02.001

Simsek, S., Ozcetin, C., Balkaya, I. (2012): Detection of polymorphism in AgB1 gene from sheep, cattle and human isolates of echinococcus granulosus by SSCP. *Vet. Parasitol.,* 184: 352 – 355. DOI: 10.1016/j.vetpar.2011.09.002

Teofanova, D., Kantzoura, V., Walker, S., Radoslavov, G., Hristov, P., Theodoropoulos, G., Bankov, I., Trudgett, A. (2011): Genetic diversity of liver flukes (*Fasciola hepatica*) from Eastern Europe. *Infect. Gen. Evol.,* 11: 109 – 115. DOI: 10.1016/j.meegid.2010.10.002

WHO (2015): Neglected tropical diseases list. Internet Access: http://www.who.int/neglected_diseases/diseases/en/

DOI 10.2478/helm-2019-0003

HELMINTHOLOGIA, 56, 2: 93 – 107, 2019

Molecular characterization and Immunodiagnostic potential of various antigenic proteins of *Fasciola gigantica* species isolated from sheep of North West Himalayan Region

J. S. DAR[1]*, B. A. GANAI[1], R. A. SHAHARDAR[2], U. R. ZARGAR[3]

[1]Centre of Research for Development, University of Kashmir, Srinagar, 190 006, *E-mail: *humjehan@gmail.com*;
[2]Department of Veterinary Parasitology, SKUAST, Kashmir, 190 006; [3]Department of Animal Sciences,
Sonwar Campus, Srinagar, 190 004

Article info

Received September 4, 2018
Accepted December 17, 2018

Summary

The control of the digenetic trematode *Fasciola gigantica* has been the major challenge in both cattle and small ruminants as there is a paucity of an effective and commercial vaccine. Thus, the accurate identification and prepatent diagnosis of *F. gigantica* is an essential prerequisite for its successful prevention and control. In the present study, the morphologically identified specimens isolated from the liver and bile ducts of sheep (*Ovis aries*) were validated through molecular data. The sequence analysis of ITS-2 of our isolates showed high degree of similarity with *F. gigantica* and *F. hepatica* using BLAST function of NCBI. The phylogenetic analysis of our isolates showed a close relationship with previously described *F. gigantica* and *F. hepatica* isolates from different countries. The antigenic profile of somatic and E/S antigens of *F. gigantica* were revealed by SDS–PAGE and immunoblotting using sera from sheep naturally infected with *F. gigantica.* By SDS-PAGE, 20 distinct bands were revealed from crude somatic fraction. Immunoblotting analysis of these proteins with positive sera exhibited 8 sero-reactive bands ranging from 14 to 97 kDa. Among these 38 and 44 kDa bands were quite specific with high diagnostic specificity and sensitivity. The E/S fraction comprised 7 distinct bands, as revealed by SDS-PAGE analysis. Immunoblotting analysis of these proteins with positive sera exhibited 6 antigenic bands ranging from 23 – 54 kDa. Among these 27 and 33 kDa were found to be quite specific with high diagnostic specificity and sensitivity. The present study concludes that the protein bands of 38 and 44 kDa in somatic fraction and 27 and 33 kDa in E/S fraction can be used for the immunodiagnostic purpose for this economically important parasite, which may also entice further studies regarding their vaccine potential.

Keywords: *Fasciola gigantica*; ITS-2; BLAST; Excretory/Secretory antigen (E/S); Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE); Immunoblotting

Introduction

Livestock industry is faced with various types of bottlenecks that affect its production and parasitic infection is one of the important factors responsible for decline of livestock industry. A large groups of parasites like protozoans, trematodes, cestodes and nematodes are known to impinge on the livestock industry, but trematodes have been found to be most disparaging, especially the gastrointestinal trematodes (Vercruysse & Claerebout, 2001; Roeber *et al.,* 2013). The members of the genus *Fasciola*, also known as liver flukes are responsible for causing a disease called 'fasciolosis' in livestock as well as humans. Because of their large size and cosmopolitan distribution, they have the global economic implications and are regarded as a concern for livestock and hu-

* – corresponding author

man health. The economic loss incurred at the global level by fasciolosis infection is estimated to be about US$ 3.2 billion annually which may be due to reduction of weight gain, draught tolerating capacity, fertility and lactation and costs associated for chemotherapy (Charlier *et al.,* 2008; Khan *et al.,* 2017). Equally important concern is the zoonotic transmission of these diseases to humans with 2.4 to 17 million people across world reported to be infected by fasciolosis and between 90 and 180 million known to be at risk of infection (Anonymous, 1995; Toledo *et al.,* 2011). Several studies have shown that *F. hepatica* and *F. gigantica* have unequal distribution. Whilst, *F. hepatica* is distributed throughout the world, *F. gigantica* has limited distribution and found only in areas of Asia and Africa. Further in the areas of geographical overlap, aspermic hybrid forms have developed making it difficult to differentiate between them. Such aspermic hybrids, which are thought to be the result of interspecific hybridization between the species of *F. hepatica* and *F. gigantica,* have been reported from Asia (Itagaki *et al.,* 2005a). Although the morphological characteristics are used as the basis to differentiate these two species of *Fasciola* (Ashrafi *et al.,* 2006), their accurate differentiation is difficult because at the extremes of their size range, individuals of one species may resemble with the other, with intermediate forms also occurring. Thus it becomes necessary to use alternative techniques like the modern molecular tools and markers for their identification and differentiation (Marcilla *et al.,* 2002; Periago *et al.,* 2004). For this purpose, a number of genes have been used which include the sequences of the first (ITS-1), the 5.8S and second (ITS-2) Internal Transcribed Spacers (ITS) of the nuclear ribosomal DNA (rDNA), 28S ribosomal ribonucleic acid (rRNA) (Adlard *et al.,* 1993; Itagaki and Tsutsumi, 1998; Marcilla *et al.,* 2002; Itagaki *et al.,* 2005a; Le *et al.,* 2008; Ichikawa and Itagaki, 2010), 18S rRNA (Karimi, 2008), mitochondrial NADH dehydrogenase I (NDI) and Cytochrome c Oxidase I (COI) genes (Hashimoto *et al.,* 1997; Itagaki *et al.,* 2005b). Further the use of novel single copy markers of nuclear genes like phosphoenolpyruvate carboxykinase (*pepck*) and DNA polymerase delta (*pold*) genes in the recent years have been developed in this direction for the accurate discrimination of *F. hepatica, F. gigantica* and their hybrid forms (Shoriki *et al.,* 2015). Diagnosis of fasciolosis is an essential prerequisite for successful prevention and control of a parasitic disease. Efficient and state-of-the-art diagnostic tools and methods are important in the disease management program. Various tools ranging from traditional parasitological techniques to modern high-end molecular and immunological tools have been employed for the purpose. The most commonly used parasitological technique includes the fecal examination for the microscopic detection of liver fluke eggs by Kato-Katz test (Peters *et al.,* 1980). However, absence of eggs in the pre-patent phase and intermittent release of eggs in mature phase makes the results unreliable (Nour Eldin *et al.,* 2004). Moreover, in coproscopic detection of liver fluke infection faces limited accuracy and greater chances of false positive results (Dorchies, 2007). Therefore, early diagnosis is essential for effective treatment of the disease (Rokni *et al.,* 2004). In this direction various immunological detection techniques have been devised which are based on the presence of antibodies against the fluke antigens (Maleewong *et al.,* 1999). Such tests allow the pre-patent detection of the liver fluke disease before egg excretion. Other immunodiagnostic methods, involve the detection of fluke antigens inside the serum or other body fluids of infected host. Such tests are more accurate and allow early diagnosis than the antibody detection methods as the antigen is present in the host's serum or fluids before the presence of antibodies (Cornelissen *et al.,* 1999). However, these methods are also beset with many disadvantages, such as cross reactions with other trematode parasites, leading to false positive results (Hillyer *et al.,* 1985). The use of Western blotting/Immunoblotting techniques in recent years, have been able to overcome the chances of cross reactions to a greater extant. Of late, the emergence of proteomics has generated many biomarkers for efficient diagnosis of diseases. The identification and characterization of various proteins (mainly the candidates for immunodiagnosis or vaccination) over last two decades have been found to be of much immunological significance (Moxon *et al.,*

Table 1. Comparison of sequence lengths and composition of ITS2 rDNA of *Fasiola gigantica* from different parts of the world with our isolate JF1.

Species	Nucleotide Composition							
	Accession No.	ITS 2 (bp)	A+T (%)	G+C (%)	A	C	G	T
F. gigantica (JF1)	MH048702	362	51.93	48.07	71	80	94	117
F. gigantica (Iran)	JN828953	349	52.15	47.85	71	76	91	111
F. gigantica (Kenya)	KP760871	363	52.07	47.93	71	80	94	118
F. gigantica (India)	KX467878	365	51.51	48.49	71	82	95	117
F. gigantica (Australia)	MF678651	362	51.93	48.07	71	80	94	117
F. gigantica (Japan)	AB207152	363	51.79	48.21	71	81	94	117
F. gigantica (Indonesia)	AB010977	362	51.93	48.07	71	80	94	117
F. gigantica (Zambia)	AB010976	362	51.93	48.07	71	80	94	117
F. gigantica (Burkina Faso)	AJ853848	363	52.07	47.93	71	80	94	118

2010; Toledo *et al.,* 2011). Keeping all this in view, the current study was designed to properly identify the species of *F. gigantica* and differentiate it from *F. hepatica* by using the morphological and molecular data. The study was also carried out to recognize the somatic and excretory/secretory antigenic profile of *F. gigantica* by immunoblotting technique using sera from sheep naturally infected with *Fasciola gigantica*.

Materials and Methods

Collection of Parasites

Adult flukes of *F. gigantica* were collected from the liver and bile ducts of naturally infected Sheep (*Ovis aries*) slaughtered for consumption at the local slaughter houses. The samples for this study were collected during 2014 and 2015 from various slaughter houses of Kashmir valley. The collected flukes were washed 3 – 4 times with phosphate buffer saline (PBS) and used immediately for antigen preparation or stored in the laboratory at -20 °C until used.

Identification of the Parasite

The flukes were fixed in formalin, stained with hematoxylin and eosin, dehydrated in series of ascending grades of ethanol, cleared in xylene and mounted in DPX. The species were then identified morphologically according to Soulsby, 1982.

Genomic DNA Isolation, Quality check and Amplification

Genomic DNA was isolated from a piece of apical and lateral portions of adult *F. gigantica* using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. DNA was eluted in 50 µl of elusion buffer (10 mM Tris, 1 mM EDTA) and kept at -20 °C until use. The quality of the DNA isolated was checked by Agarose gel electrophoresis using 0.8 % agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide.

PCR was used to amplify the nuclear ITS-2 of ribosomal DNA by using the Primers BD1 (forward: 5'- GTCGTAACAAGGTTTCCGTA -3') and BD2 (reverse: 5'- TATGCTTAAATTCAGCGGGT -3'). PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2mM each dNTPs (dATP, dGTP, dCTP and dTTP), 1 µl DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3 % DMSO, 0.5M Betaine, 5pM of forward and reverse primers. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) with initiation at 94 °C for 5 min, followed by 35 cycles including denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 50 s; a final extension step consisting of incubation at 72 °C for 10 min was included. The PCR products were checked in 1.2 % agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system.

Sequencing and Phylogenetic analysis

After amplification of the DNA samples, the amplified products were sent to Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram, Kerala for sequencing. The sequences obtained from each primer were assembled and edited in Bioedit sequence alignment editor (Hall 1999). All the edited sequences were aligned in MEGA 7.0 [30], using the clustalW algorithm (Kumar *et al.,* 2016), in order to trace individual mutations. The sequences were identified using BLAST function from NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). Additional sequences of *F. hepatica* and *F. gigantica* from different geographical regions were retrieved from GenBank for sequence alignment and phylogenetic tree construction. Phylogenetic trees were constructed by Minimum Evolution method (Rzhetsky and Nei, 1992) in MEGA 7.0 (Kumar *et al.,* 2016) based on Tamura and Nei model.

Preparation of crude Somatic and excretory-secretory (E/S) proteins

Somatic proteins were extracted by the methodology described by Anuracpreeda *et al.,* (2009). Protein extracts were obtained by homogenization of 5 flukes in 5 ml of ice cold lysis buffer (0.01 M phosphate buffer saline (PBS), pH 7.2, containing 10 mM Tris–HCl, 150 mM NaCl, 0.5 % Triton X-100, 10 mM EDTA, 1 mM PMSF and 100 µl of cocktail of protease inhibitors) using tissue homogenizer. Homogenization was carried out for 10 minutes at 1300 rpm taking pause of 2 minutes after every one minute of homoginization. The supernatant protein fraction was concentrated using chilled acetone.

Table 2. Comparison of sequence lengths and composition of ITS2 rDNA of *Fasiola hepatica* from different parts of the world with our isolate JF4.

Species	Nucleotide Composition							
	Accession No.	ITS 2 (bp)	A+T (%)	G+C (%)	A	C	G	T
F. hepatica (JF4)	MH048706	363	51.79	48.21	70	80	95	118
F. hepatica (Australia)	MF678650	363	51.79	48.21	70	80	95	118
F. hepatica (Turkey)	JN585288	362	51.38	48.62	70	81	95	116
F. hepatica (Egypt)	AB553720	363	51.79	48.21	70	80	95	118
F. hepatica (Japan)	AB010978	362	51.66	48.34	70	80	95	117

Excretory-Secretory (E/S) proteins were prepared from living flukes according to Phiri *et al.,* (2006) with slight modifications. The adult worms of *F. gigantica* were collected from the liver and bile ducts and washed 3 times in 0.01 M phosphate buffered saline (PBS), pH 7.4, at room temperature. The cleaned worms were then incubated (40 worms per 100 ml) in RPMI-1640 medium containing 2 mM phenylmethanesulfonyl fluoride (PMSF), 100 IU of penicillin and 100 µg of streptomycin per milliliter of medium for about 3 hours at 37 °C. After incubation, the worms were removed from the medium and the suspension containing the E/S proteins was centrifuged at 13000 rpm for 30 minutes at 4°C. The supernatant was collected, aliquoted and stored at -20°C. The protein concentrations of both fractions were measured according to Bradford (1976).

Collection of sera samples

Blood samples were collected from local abattoirs at the time of slaughtering of sheep with the mono-specific infections of *F. gigantica* and from uninfected negative control sheep. Blood sample was also collected from sheep infected with *Paramphistomum* spp. The infections were ascertained by Postmortem examinations and by coprological techniques (Thienpont *et al.,* 1979; Olaechea *et al.,* 1990). Sera were obtained from each clotted blood samples by centrifugation at 4000 rpm for 10 minutes, and stored at -20°C.

SDS-PAGE and Immunoblotting

Somatic and E/S proteins of *F. gigantica* were separated by SDS-PAGE as described by Laemmli (1970). The SDS-PAGE antigen separation was done under reducing conditions in gradient polyacrylamide gel cast with 12 % separating and 6 % stacking gel mixture. The electrophoresis was carried out in Mini Protean II electrophoresis apparatus (Bio-Rad, Hercules, CA) at 90 V for about 3 – 4 hours. Gels were stained with 0.05 % Coomassie brilliant blue. The molecular weights of proteins were then determined by comparing their migration distance against that of a known molecular marker. After SDS-PAGE, the unstained gels were transferred electrophoretically onto a nitrocellulose sheet using a transfer blot apparatus. Nitrocellulose containing transferred sample strips were incubated in a blocking solution (I % skimmed milk and 0.1 % Tween 20 in 100 mM PBS, pH 7.4) overnight at 4°C. The strips were then incubated with sera containing test antibodies. All sera were diluted 1:1000 in TBS and incubated at 4°C overnight with gentle shaking. Following 3 PBS washes to remove unbound antibodies, the nitrocellulose sheets were then incubated for 1 hour in horseradish peroxidase conjugate anti-IgG antibodies. Unbound conjugate was removed by 3 PBS washes before the addition of substrate solution containing DAB (3,3'- Diaminobenzidine). Gels were then visualized under Bio Rad gel documentation system.

Evaluation of the diagnostic sensitivity of above antigens*:*

The diagnostic sensitivity, specificity, accuracy and predictive values of various antigens were tested with the sera from 12 sheep with natural infections of *Fasciola gigantica,* 6 uninfected hosts, together with 6 sera from sheep with *Paramphistomum* infection. Each of these parameters was calculated using following formulas:

Sensitivity= [A/(A+C)]×100

Specificity= [D/(B+D)] x 100.

Accuracy = [(A+D)/(A+B+C+D)] × 100

Positive predictive value= [A/(A+B)] x 100.

Negative predictive value= [D/(C+D)] x 100.

Where;

A= number of true positive,

B= number of false positive,

C= number of false negative,

D= number of true negative.

The primary data of immunoblotting are as follows:

True positive = number of proven infected samples that show positive result.

True negative = number of control samples (other parasitoses and healthy controls)
that show negative result.

False positive = number of control samples that show positive result.

False negative = number of proven infected samples that show negative result.

Ethical approval

The conducted research is not related to either human or animal use.

Results

Morphological identification of *F. gigantica* was carried out on the basis of size and shape of fluke, presence of shoulders and position of anterior and posterior sucker. Adult flukes of *F. gigantica* were found to be larger in size (up to 60 mm) than *F. hepatica.* The body shape was found to be dorso-ventrally flat or leaf

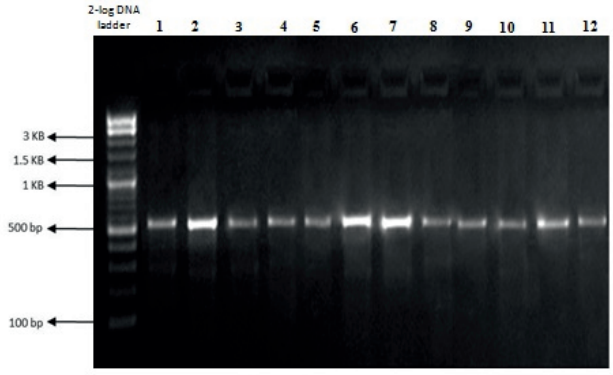

Fig. 1. Agarose gel electrophoresis of ITS-2 rDNA. Lanes 1 – 7 (*F. gigantica*) and lanes 8 – 12 (*F. hepatica*).

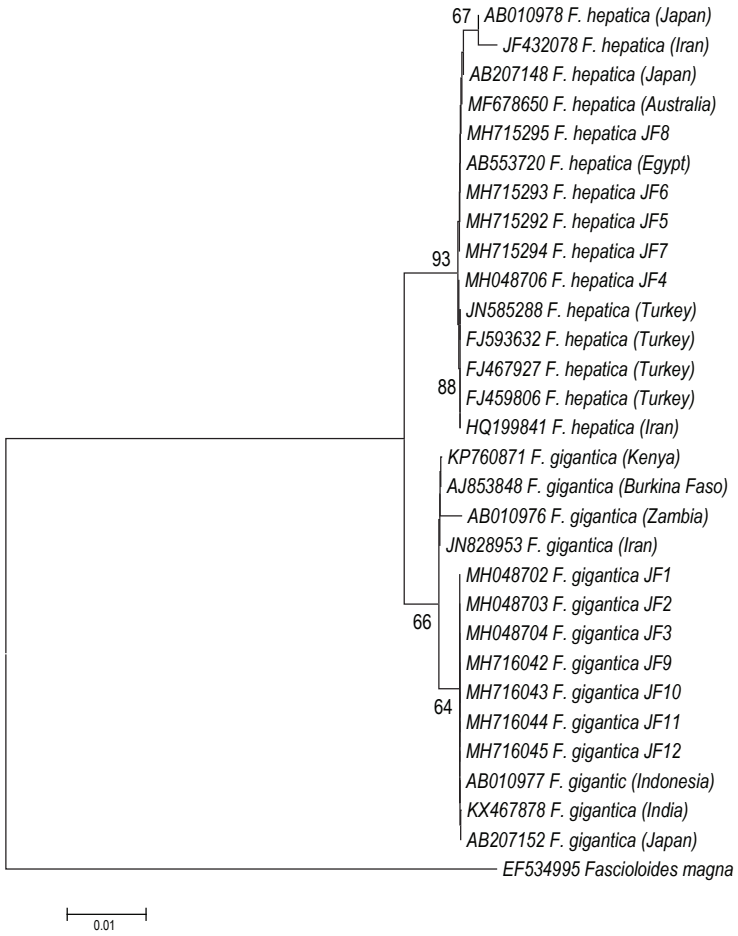

Fig. 2. Minimum Evolution phylogenic tree, the bootstrap test (10000 replicates), based on ITS-2 gene of *F. gigantica* and *F. hepatica*, *Fascioloides magna* (EF534995) designated as outgroup. The analysis involved 30 nucleotide sequences. All ambiguous positions were removed from each sequence pair. There were a total of 367 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

like and possess conical anterior end followed by the shoulders. The body was found to be covered by the tegument armored with backwardly projecting spines. Ventral sucker also known as acetabulum was found to be larger than the oral sucker.

Molecular Characterization

During the present investigation, liver fluke species viz. *Fasciola gigantica* and *F. hepatica* were obtained and molecular data confirmed and validated the morpho-taxonomic characterization. Among these, seven isolates viz JF1-JF3, JF9-JF12 were found close to topotype population of *Fasciola gigantica* and five isolates (JF4-JF8) showed similarity with *F. hepatica.* The PCR amplification of ITS-2 using Primers BD1 (forward: 5'- GTCGTAACAAGGTTTCCGTA -3') and BD2 (reverse: 5'- TATGCTTAAATTCAGCGGGT -3') yielded fragments of 550 bp for both the species of *Fasciola* (Fig. 1). The sequence analysis showed that ITS2 rDNA had a length of 362 base pair in case of *F. hepatica* isolates and 363 base pair in *F. gigantica* isolates and, along with ITS2 were partial sequences of 5.8s and 28s rDNA sequences. The annotated sequences of these isolates were submitted to NCBI via Banklt with accession numbers: MH048702, MH048703, MH048704, and MH716042-MH716045 for seven *F. gigantica* isolates, MH048706 and MH715292-MH715295 for five *F. hepatica* isolates. The BLAST analysis of rDNA ITS2 sequences of JF1-JF3, JF9-JF12 isolates showed 97 – 100 % similarity with *F. gigantica*, while the sequences of ITS2 rDNA of JF4-JF8 isolates showed 97 – 100 % similarity with *F. hepatica*.

Phylogenetic trees were constructed by comparing ITS2 sequences of this study with those of other liver fluke species deposited in the GenBank from across the world. Minimum evolution tree was used to construct phylogeny. *F. gigantica* and *F. hepatica* were separated in two clusters. Reliable grouping among ITS2 sequences of *F. gigantica* and *F. hepatica* from the current study and those from Australia, Indonesia, Japan, Burkina Faso, Kenya, Zambia, Iran, Egypt, Turkey and India are shown in Fig. 2.

The phylogenetic analysis of the *Fasciola* species based on ITS-2 regions by Minimum evolution tree showed a clear monophyly of the group formed by the present *F. gigantica* isolates and previ-

Table 3: Pairwise distances of the ITS-2 region of *Fasciola* Spp. from Kashmir India, compared with other sequences submitted to GenBank..

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 **MH048702 *F. gigantica* isolate JF1**		0	0	0	0	1	1	1	2	5	5	5	5	5	5	5	5	5	6	39
2 KX467878 *F. gigantica* (India)	100		0	0	0	1	1	1	2	5	5	5	5	5	5	5	5	5	8	39
3 MF678651 *F. gigantica* (Australia)	100	100		0	0	1	1	1	2	5	5	5	5	5	5	5	5	5	6	39
4 AB207152 *F. gigantica* (Japan)	100	100	100		0	1	1	1	2	5	5	5	5	5	5	5	5	5	7	39
5 AB010977 *F. gigantica* (Indonesia)	100	100	100	100		1	1	1	2	5	5	5	5	5	5	5	5	5	6	39
6 KP760871 *F. gigantica* (Kenya)	100	100	100	100	100		0	0	1	4	4	4	4	4	4	4	4	4	8	39
7 JN828953 *F. gigantica* (Iran)	100	100	100	100	100	100		0	1	4	4	4	4	4	4	4	4	4	4	37
8 AJ853848 *F. gigantica* (Burkina Faso)	100	100	100	100	100	100	100		1	4	4	4	4	4	4	4	4	4	6	39
9 AB010976 *F. gigantica* (Zambia)	100	100	100	100	100	100	100	100		5	5	5	5	5	5	5	5	5	6	40
10 **MH048706 *F. hepatica* isolate JF4**	99	99	99	99	99	99	99	99	99		0	0	0	0	0	0	0	0	1	39
11 MF678650 *F. hepatica* (Australia)	99	99	99	99	99	99	99	99	99	100		0	0	0	0	0	0	0	1	39
12 JN585288 *F. hepatica* (Turkey)	99	99	99	99	99	99	99	99	99	100	100		0	0	0	0	0	0	3	39
13 FJ593632 *F. hepatica* (Turkey)	99	99	99	99	99	99	99	99	99	100	100	100		0	0	0	0	0	3	39
14 FJ467927 *F. hepatica* (Turkey)	99	99	99	99	99	99	99	99	99	100	100	100	100		0	0	0	0	3	39
15 FJ459806 *F. hepatica* (Turkey)	99	99	99	99	99	99	99	99	99	100	100	100	100	100		0	0	0	3	39
16 AB553720 *F. hepatica* (Egypt)	99	99	99	99	99	99	99	99	99	100	100	100	100	100	100		0	0	1	39
17 AB010978 *F. hepatica* (Japan)	99	99	99	99	99	99	99	99	99	100	100	100	100	100	100	100		0	0	39
18 HQ199841 *F. hepatica* (Iran)	99	99	99	99	99	99	99	99	99	100	100	100	100	100	100	100	100		3	39
19 JF432078 *F. hepatica* (Iran)	99	99	99	99	99	99	99	99	99	100	100	100	100	100	100	100	100	100		41
20 EF534995 *Fascioloidesmagna*	93	93	93	93	93	93	93	93	93	93	93	93	93	93	93	93	93	93	100	

Below diagonal: percentage similarity; above diagonal: total character differences.

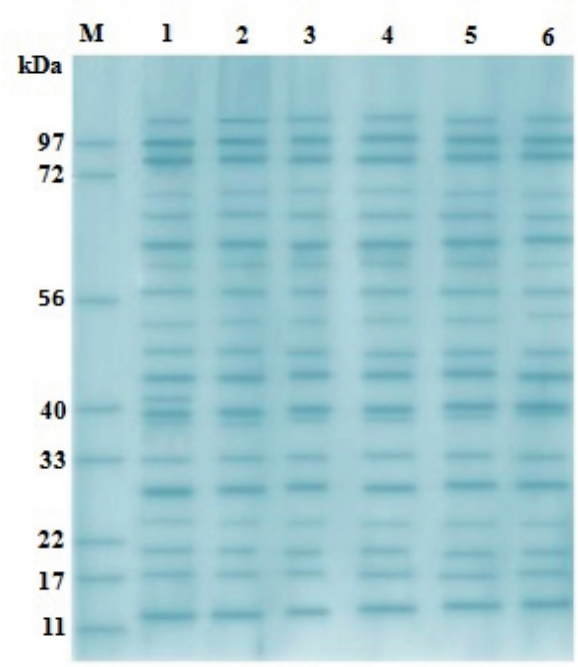

Fig. 3. Commassie Brilliant Blue stained protein profile of whole worm extract of *Fasciola gigantica* from different sheep livers (lane L1-L6), molecular weight marker (M).

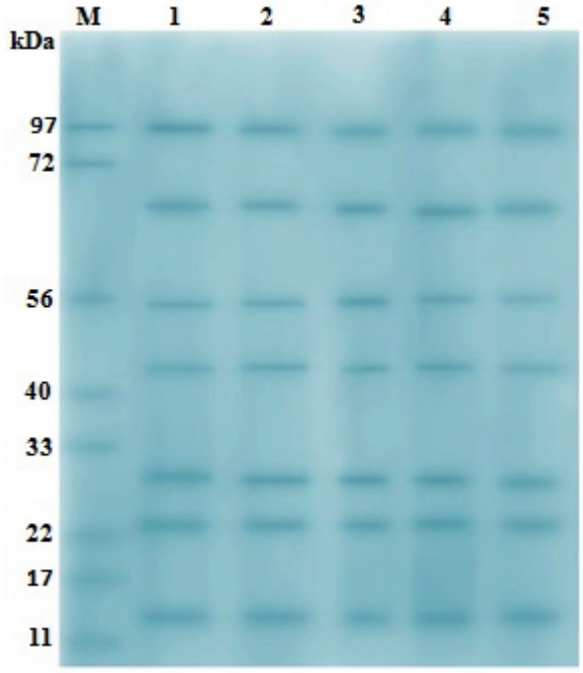

Fig. 4. Commassie Brilliant Blue stained profile of Excretory/ Secretory antigens of *Fasciola gigantica* from different sheep livers (lane L1-L5), molecular weight marker (M).

ously described *F. gigantica* isolates from different countries while the current studied isolates of *F. hepatica* formed a clade with *F. hepatica* reported from different countries. *Fascioloides magma* (EF534995) was used as outgroup taxa and bootstrap values are shown next to clades (Fig. 2). By comparing the nucleotide sequence of ITS-2, the two species of *Fasciola* were found to differ at six nucleotide sites viz: 210, 234, 273, 279, 330 and 337. These include four transitions (CT) at 210, 234, 273 and 279; one indel at 330; and one transition (AG) at 337. The nucleotide compositions of *F. gigantica* and *F. hepatica* current isolates and other reported from other countries are summed in Table 1 and Table 2. The distance matrix analysis carried by p-distance and maximum composite likelihood method of MEGA 7 showed that the present *F. gigantica* isolates showed 100 % similarity and zero total character difference with already described species of *F. gigantica*, as far as their ITS-2 rDNA sequences are concerned while with *F. hepatica,* 5 – 6 bp differences were recorded. On the contrary, *F. gigantica* isolates showed maximum similitude (100 % similarity) and zero total character difference with already described species of *F. hepatica*; however, with *F. gigantica* 5 – 6 bp differences were noticed (Table 3).

SDS-PAGE and Immunoblotting

Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE, 6 % stacking and 12 % resolving gel) protein profile of *F. gigantica* crude extract under reducing conditions, revealed 20 bands with molecular weight ranging from 14 – 110 kDa (Fig. 3).

Simillarly Electrophoretic protein profile of total E/S antigen under reducing conditions revealed 7 polypeptide bands in the range of 14 – 97 kDa. These bands were detected at 14, 23, 27, 44, 54, 66 and 97 kDa (Fig. 4).

Western blotting of the crude somatic protein profile of *F. gigantica* with the sera of positive animals showed eight immunoreactive bands with molecular weights of 14, 23, 27, 38, 44, 66, 84 and 97 kDa (Fig. 5). Out of the 12 sera from *F. gigantica* infected sheep tested, 38 and 44 kDa proteins were found to give a consistent reaction with all the 12 sera samples and 84 kDa band was detected by 10 sera samples while as, the protein bands of 14 kDa was detected by 7, 23 and 27 kDa by 8, and 66 and 97 kDa by 9 sera samples. Further the cross reactivity of the somatic antigens of *F. gigantica* was determined by immunoblotting with six sera samples from sheep infected with paramphistomosis. With six sera samples from sheep infected with paramphistomosis, 38 and 44 kDa proteins bands were not detected by the any sera, the protein bands of 23, 27, 66, 84 kDa and 97 kDa were found to cross react with the 5, 3, 4, 1 and 4 sera samples of paramphistomosis infected sera respectively. 66 and 97 kDa proteins were even detected with the 2 sera samples out of 6 negative sera tested. The diagnostic sensitivity, specificity, accuracy and predictive values for somatic immunogenic proteins are given in Table 4. Therefore from the data, we conclude that 38 and 44 kDa proteins are quite specific for *F. gigantica* in crude antigen

Six immunodominant bands with molecular weights of 23, 27, 29, 33, 44 and 54 kDa were revealed by blotting the E/S fraction

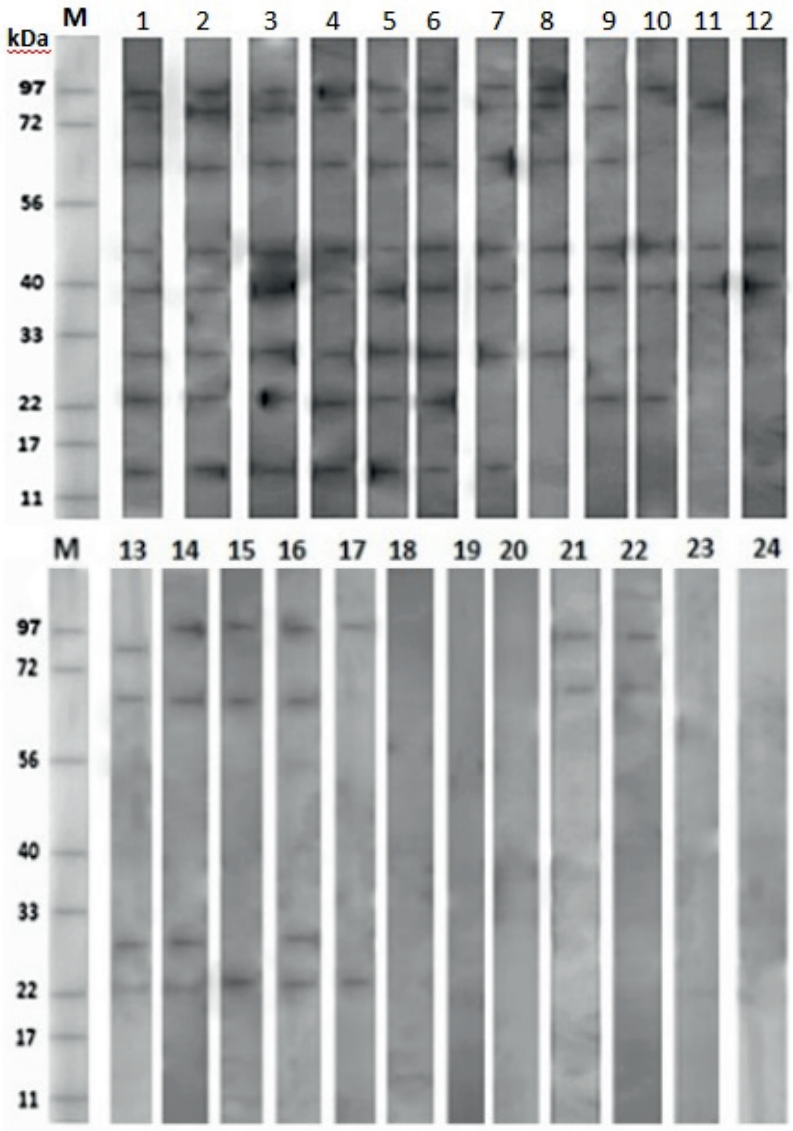

Fig. 5. Western blot analysis of somatic antigens of *Fasciola gigantica* against sera samples M: Molecular weight marker, 1-12: Positive sera of *Fasciola gigantica*, 13-18: Positive sera of *Paramphistomum sp*., 19-24: Negative control sera.

of proteins with the 12 sera samples of infected sheep with clinically diagnosed and parasitologically confirmed fasciolosis (Fig. 6). The protein bands of 27 and 33 kDa were found to give a consistent reaction with all 12 positive sera samples tested. Other protein bands with M.W of 23, 29, 54 and 44 kDa were detected only in 8, 10, 7 and 9 sera samples respectively out of total 12 positive sera samples. Moreover the bands of 23, 33 and 54 kDa were found to cross react with 3, 2 and 4 respectively out of 6 sera of paramphistomosis infected sheep. No band was observed when immunoblotted with 6 uninfected negative control sera. The diagnostic sensitivity, specificity, accuracy and predictive values for E/S immunogenic proteins are given in Table 5. Thus we conclude that the protein bands of 27 and 33 kDa are quite specific for *F. gigantica* in E/S antigen with high diagnostic specificity and sensitivity.

Discussion

The parasitic burden of trematode parasites has been a great challenge for veterinary practitioners in this part of world, and therefore it was felt to investigate one of the most economically important liver parasite i'e *Fasciola gigantica*. The overarching aims of the present investigation were to characterize this economically important parasite morphologically and molecularly and to identify its specific somatic and E/S antigens through Immunoblotting.

The use of ITS-2 gene in the present study for the species identification of liver flukes has been confirmed from a number of previous findings throughout the world (Adlard *et al.,* 1993; Ali *et al.,* 2008). Further the nucleotide sequences from mitochondrial DNA (NDI and COI) in addition to ITS-1 and ITS-2 genes of ribosomal

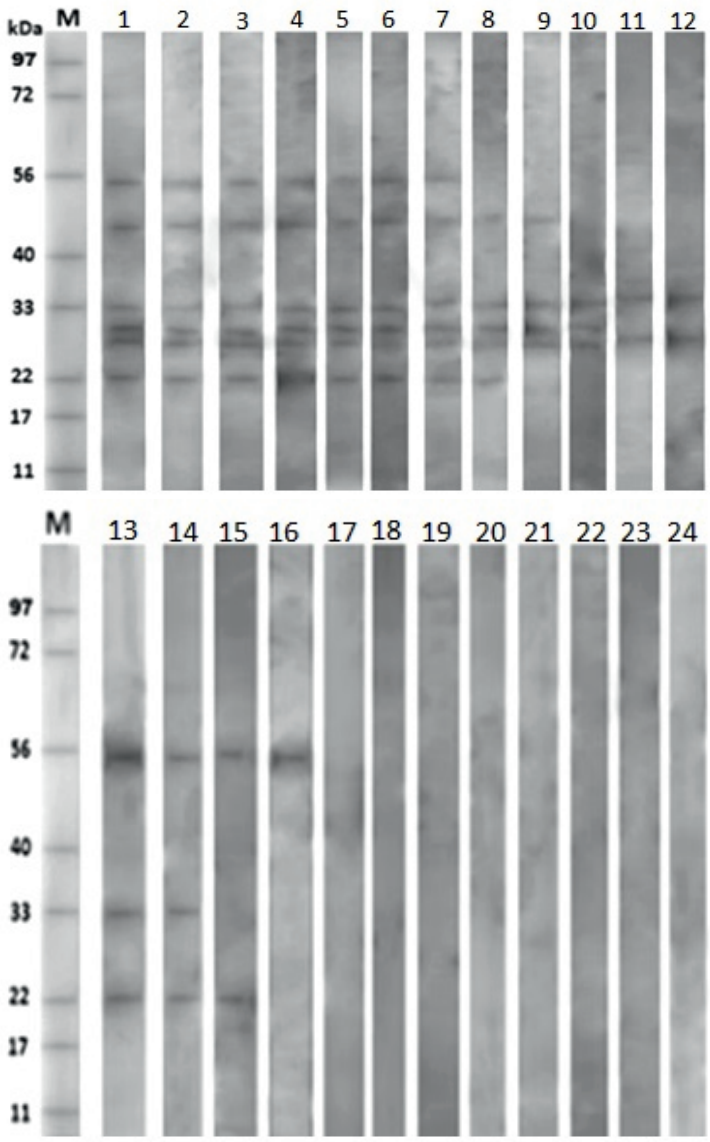

Fig. 6. Western blot analysis of Excretory/secretory antigens of *Fasciola gigantica* against sera samples M: Molecular weight marker, 1-12: Positive sera of *Fasciola gigantica*, 13-18: Positive sera of *Paramphistomum sp*., 19-24: Negative control sera.

DNA have been effectively used for proper identification and differentiation of fasciolids as well as elucidating their origin and source of infection (Moghaddam *et al.,* 2004; Amor *et al.,* 2011; Itagaki *et al.,* 2005b). By comparing the nucleotide sequence of ITS-2, the two species of *Fasciola* were found to differ at six nucleotide sites *viz*; 210, 234, 273, 279, 330 and 337. These include four transitions (CT) at 210, 234, 273 and 279; one indel at 330; and one transition (AG) at 337. These results were in accordance with the studies of Adlard *et al.,* 1993, Agatsuma *et al.,* 2000, Huang *et al.,* 2004, Ali *et al.,* 2008 and Raina *et al.,* 2015, who also revealed six nucleotide differences in the ITS-2 between *F. hepatica* and *F. gigantica.* However, differences in seven nucleotide sites of ITS-2 between the two species of *Fasciola* have been reported by Choe *et al.,* 2011 and Shafei *et al.,* 2014. Similar type of study was carried out by Galavani *et al*., 2016 from West Azerbaijan Province Iran, who reported 100 % similarity of 1081 bp fragment of ITS-1 (428 bp), 5.8S (158 bp) and ITS-2 (366 bp) of their study isolates with *F. hepatica*. In their study, Only *F. hepatica* species was found to be distributed among sheep and cattle in West Azerbaijan Province Iran as the nucleotide sequence divergence for ITS2 among the isolates was found to be negligible or nil. The molecular characterization of *Fasciola* species collected from sheep in the present study confirmed the findings of Sharma *et al.,* 1989, who reported the presence of both *F. hepatica* and *F. gigantica* from the sheep of Kashmir valley. The phylogentic analysis of *Fasciola* species based on their ITS-2 sequences using Mini-

Table 4. Sensitivity, specificity, accuracy, Positive and negative predictive values of most prevalent positive bands using *Fasciola gigantica* somatic antigens

Test band (kDa)	Sensitivity (%)	Specificity (%)	Accuracy (%)	Predictive Value	
				Positive (%)	Negative (%)
14	58.33	100	79.16	100	70.59
23	66.67	58.33	62.5	61.54	63.64
27	66.67	75	70.83	72.73	69.23
38	100	100	100	100	100
44	100	100	100	100	100
66	75	50	62.5	60	66.67
84	83.33	91.67	87.5	90.91	84.62
97	75	66.67	62.5	69.23	72.73

mum Evolution method showed that the seven isolates, JF1-JF3, JF9-JF12 formed the clade with *Fasciola gigantica* isolates from different country while five isolates JF4-JF8 formed the clade with *Fasciola hepatica* isolates from different country. ITS-2 sequence of *Fasciola gigantica* isolates in our study formed a single cluster with the *Fasciola gigantica* isolates from India, Indonesia and Japan with 100 % similarity. However the sequences obtained from GenBank from Kenya, Zambia, Iran and Burkina Faso got separately clustered. Similarly the ITS-2 sequence of *Fasciola hepatica* in our study formed a single cluster with the *Fasciola hepatica* isolates from Australia, Egypt, Turkey, Iran and Japan with 100 % similarity. Similar type of study by Prasad et al., 2008 showed close relationship of *Fasciola gigantica* isolates from Assam India with the *Fasciola gigantica* isolates from China, Indonesia, Japan, Egypt and Zambia with significant bootstrap values.

The present study was also focused to identify the somatic and E/S antigens of *F. gigantica* which can be used for immunodiagnostic purpose. In our studies with *F. gigantica*, protein profiling of crude somatic antigens by SDS-PAGE revealed 20 bands with M.Ws ranging from 14 – 110. Similar studies were carried out on this fluke by number of authors who revealed different band patters in their somatic extracts, however most of the distinct bands were found to be in the similar ranges. The study carried out by Gupta *et al.,* (2003) revealed six polypeptide bands between the molecular weights of 27.7 – 37.5 kDa, while Meshgi *et al.,* (2008) revealed 11 major bands having range between 18 – 68 kDa for somatic antigens of *F. gigantica.* In similar kind of study, Allam *et al.,* (2002) reported 5 bands between 27 – 57.6 kDa, Gonec *et al.,* (2004) reported 7 bands between 6.5 – 205 kDa, Upadhyay and Kumar (2002) reported 7 bands between 16 – 62 kDa, Hassan *et al.,* (2014) reported 15 bands between 14 – 165 kDa. Along the similar lines, Sobhon *et al.,* (1996) by SDS-PAGE profiling of the homogenized whole body of *F. gigantica* analyzed approximately 21 detectable bands with molecular weights ranging from 17 – 110 kDa, while as the tegument antigens (extracted by Triton X-100) electrophoresed only into eleven bands with M.Ws of 97, 86, 66, 64, 58, 54, 47, 38, 35, 19, and 17 kDa. Yokananth *et al.,* (2005) by electrophoretic separation of somatic antigens of *F. gigantica*

Table 5. Sensitivity, specificity, accuracy, Positive and negative predictive values of most prevalent positive bands using *Fasciola gigantica* E/S antigens.

Test band (kDa)	Sensitivity (%)	Specificity (%)	Accuracy (%)	Predictive Value	
				Positive (%)	Negative (%)
23	66.67	75	70.83	72.73	69.23
27	100	100	100	100	100
29	83.33	100	91.66	100	85.71
33	100	83.33	91.66	85.71	100
44	75	100	87.5	100	80
54	83.83	66.67	75	71.43	80

revealed 20 protein bands with molecular weights ranging from 14 – 156 kDa. Maleewong *et al.,*(1997) while working on human fasciolosis, revealed 22 polypeptide bands with molecular weight ranging from 14.4 to 94 kDa in the somatic extract of *F. gigantica* by SDS-PAGE. The number and molecular weight range of protein profile of whole worm extract of *F. gigantica* in our study was similar to the closely related species. For example, the study carried out by Cervi *et al.,* 1992 in case of *F. hepatica* revealed the somatic protein band pattern within the range of 14 – 94 kDa.

Protein profiling of E/S fraction of *F. gigantica* by SDS-PAGE revealed 7 polypeptide bands in the range of 14 – 97 kDa. These bands were detected at 14, 23, 27, 44, 54, 66 and 97 kDa. By SDS-PAGE analysis, Latchumikanthan *et al.,* (2012) reported 7 protein bands with molecular weights of 23, 25, 28, 43, 47, 52 and 66 kDa for total E/S antigen of *F. gigantica* which is similar to no. of bands in our results though the M.Ws of bands differs. The bands of 27, 44 and 54 kDa in our results may correspond to 28, 43 and 52 kDa bands in their study. The study carried out by Intapan *et al.,* (1998) on the protein profiling of E/S product of *F. gigantica* by SDS-PAGE analysis revealed six bands in the with the molecular weight ranging from less than 14.4 to 65 kDa, the band patterns of most of these bands were similar to our study. Along the similar lines, Goreish *et al.,* (2008) in their studies on protein profiling of somatic and E/S antigens of *F. gigantic* revealed that E/S antigens resolved in fewer bands compared to somatic extracts which is in line with our studies.

Immunoblotting test of resolved somatic bands with the sera of positive sheep for fasciolosis revealed the unique protein bands of 38 and 44 kDa, that were found to be quite specific for *F. gigantica* in crude antigen, as they were found to give consistent reaction with all the sera samples of positive sheep and were not cross reacting with sera of *Paramphistomum* infected and negative control sheep. Thus the protein bands of 38 and 44 kDa somatic antigens can serve as potential candidates for immunodiagnosis for fasciolosis. The antigenic band of 44 kDa was unique in our study, which was not found in early studies and needs further characterization to evaluate its immunodiagnostic potential. Similar study carried out by Maleewong *et al.,*(1997) on human fasciolosis revealed 13 bands with molecular bands ranging from less than 14.4 kDa to more than 94 kDa by immunoblotting analysis with the sera of positive patients for fasciolosis. One antigenic component of 38 kDa was found to give consistent reaction with the sera of all positive patients. Our study was partially in agreement with Maleewong *et al.,* (1997) in that we also found 38 kDa along with 44 kDa protein bands quite specific for *F. gigantica* in somatic antigen. However the study carried out by Yokananth *et al*., (2005) showed the bands of molecular weight 28 and 34 kDa in crude somatic antigens of *F. gigantica* were recognized by the sera of positive animals and were considered highly specific immunodiagnostic antigens, which in our studies were found to be less specific antigens. Similar studies on *F. hepatica* by Gonenc *et al.,* (2004) showed protein bands in the molecular weight range of 24, 33, 35, 44 – 55, and 66 kDa by immunoblotting with the sera of positive sheep for *F. hepatica*. Further the studies carried out by Santiago *et al.,* (1986) in case of *F. hepatica* reported that the sera from experimentally infected rabbits recognized the major somatic antigens at 23–28 kDa and 33, 39, 52, 58, 84 and 120 kDa, While Santiago and Hillyer, (1988) reported the major somatic antigenic bands at 69, 64 and 56 kDa by Immunoblotting of somatic antigens with the sera of positive sheep and cattle.

In our study with the Excretory/Secretory protein fraction of *F. gigantica*, 6 immunodominant bands with molecular weights of 23, 27, 29, 33, 44 and 54 kDa were revealed by immunoblotting, among these the protein bands of 27 and 33 kDa were found to give a consistent reaction with all sera samples and may be considered quite specific for *F. gigantica* in E/S antigen with high diagnostic specificity and sensitivity. Thus the protein bands of 27 and 33 kDa E/S antigens can serve as potential candidates for immunodiagnosis for fasciolosis. Our study related to revelation of 27 and 33 kDa specific bands in the E/S antigens of *F. gigantica* support and augment the findings of Intapan *et al.,* (1998), who reported the prominent antigenic band of 27 kDa, was able to react consistently with sera of most positive patients with fasciolosis. In the study carried out by of Khabisi *et al.,* (2016), the polyclonal antibodies raised in rabbit against *F. hepatica* and *F. gigantica* E/S antigen reacted with five protein bands with molecular weights of 25, 27, 29, 62 and 67 kDa. By using a specific rabbit antiserum or the sera from cattle naturally infected with fasciolosis against the E/S products of *F. gigantica,* Attallah *et al.,* (2002) revealed the presence of a highly reactive antigen with molecular weight of 26 – 28 kDa. Similarly, Intapan *et al.,* (2003) observed 100 % sensitivity and 97.4 % specificity of 27 kDa E/S antigen of *F. gigantica* in the detection of human fasciolosis. Similar antigenic band patterns were also reported from the E/S protein fractions of *F. hepatica*. For example, by using enzyme linked immunotransfer blot probed by sera from *F. hepatica* infected horses and pigs, Gorman *et al.,* (1997) reported the immunoreactive bands at 14 – 19, 22 – 30, 35 – 37 and 40 – 42 in E/S antigens. According to their studies, the protein bands in the range of 22–33 kDa could be the potential candidates for immunodiagnosis of fasciolosis in horses and pigs. Similar studies carried out by Sampaio-Silva *et al.,* (1996) in case of human fasciolosis revealed that the protein bands of 25 and 27 kDa are highly specific for the E/S antigens of *F. hepatica* and could have high immunodiagnostic potential.

A number of other studies also detected a 27 kDa protein band as an immunodominant band of both somatic and E/S of *Fasciola* species (Santiago and Hillyer 1988; Attallah *et al.,* 2002; Dixit *et al.,* 2008; Kamel *et al.,* 2013). However the studies of Sampaio Silva *et al.,* (1996), Hammami *et al.,* (1997) and Rokni *et al.,* (2004), revealed the protein band of 29 kDa molecular weight as the principle band of the E/S antigen of *Fasciola* species for the serodiagnosis of fasciolosis. This 29 kDa protein band may correspond to cysteine-L-proteinase which was isolated and identified by Dalton *et al.,* (1996) and separated from the E/S products of *F. he-*

patica. Furthermore, certain important enzymatic components, like haemoglobinase (Coles and Rubano, 1988), glutathione-S-transferase (Hillyer *et al.,* 1992) and cysteine-L-proteinases of *F. hepatica* (Simth *et al.,* 1993; Dowd *et al.,* 1994) have been identified within the molecular weight range of 27.5 to 29 kDa in E/S products of the parasite. The Cysteine proteinases in the E/S products are recognized as significant proteins which are known to play role in the biological and immune-modulatory functions in the juvenile and adult flukes, and hence has became the focus of research in last few decades. Moreover they are known to induce high protective immunity in experimentally infected cattle (Dalton *et al.,* 1996).

Excretory-secretory (E/S) antigens have proved to be more useful for immunodiagnostic purpose as well as for protection against future infections than the somatic proteins (Dalton, J. and Heffernan, M. 1989; Parkhouse *et al.,* 1987). It is because E/S antigens more commonly get in touch with the host's immune system than somatic antigens, because the parasite excretes the content of the intestine like cathepsins and other enzymes with cytolytic activities. These enzymes degrade tissues and facilitate the invasion and migration of the parasite, and induce a stronger humoral immune response.

Different workers have showed different number of protein bands by SDS-PAGE and variable number of immunodominant bands by western blotting which could be attributed to existence of genetic variability in different isolates from different host species, geographical variations, use of different extraction buffers and handling errors while homogenization and protein extraction process can be counted too. The other reason could be the presence of both endogenous and host derived proteins primarily during isolation of flukes from thawed livers as suggested by De Vera *et al.,* (2009). Moreover, the pooled extract of grouped flukes based on morphological characters may have included potential hybrid within the supposedly *F. hepatica* and *F. gigantica* samples. No matter the difference exists in the number of protein bands or molecular weights of somatic and E/S polypeptides of *Fasciola* spp., the findings of various researchers suggest existence of antigens with promising diagnostic value in human and animals.

Conclusions

The results of the present study revealed the significant nucleotide difference in the ITS-2 of ribosomal DNA between *F. hepatica* and *F. gigantica*. Moreover the results revealed some unique antigenic profiles in the somatic and E/S fractions of *F. gigantica,* which could serve as potential candidates for immunodiagnosis. These proteins however, need to be further characterized in order to ascertain their therapeutic potential as vaccine candidates. This study is also important as veterinarians have raised their concern with regard to plasticity in various economically important parasites due to altered environmental conditions, and as such there is need to study various parasite groups in different geographical regions.

Acknowledgements

Financial support from University Grants Commission (UGC), India for providing fellowship to 1st Author in the form of Junior Fellowship is acknowledged.

Conflict of Interest

Authors state no conflict of interest.

References

Adlard, R.D., Barker, S.C., Blair, D., Cribb, T.H. (1993). Comparison of the second internal transcribed spacer (ribosomal DNA) from populations and species of Fasciolidae (Digenea). *Int. J. Parasitol.*, 23: 422 – 425. DOI: 10.1016/0020-7519(93)90022-Q

Agatsuma, T., Arakawa, Y., Iwagami, M., Honzako, Y., Cahyaningsih, U., Kang, S.Y., Hong, S.J. (2000). Molecular evidence of natural hybridization between *Fasciola hepatica* and *F. gigantica*. *Parasitol. Int.*, 49: 231 – 238. DOI: 10.1016/S1383-5769(00)00051-9

Ali, H., Ai, L., Song, H.Q., Ali, S., Lin, R.Q., Seyni, B., Issa, G., Zhu, X.Q. (2008). Genetic characterisation of *Fasciola* samples from different host species and geographical localities revealed the existence of *F. hepatica* and *F. gigantica* in Niger. *Parasitol. Res.*, 102(5): 1021 – 1024. DOI: 10.1007/s00436-007-0870-7

Allam, A.F., El-Agamy, E-S., Helmy, M.H. (2002). Molecular and immunological characterization of *Fasciola* species. *Br. J. Biomed. Sci.*, 59: 191 – 195

Amor, N., Halajian, A., Farjallah, S., Merella, P., Said, K., Slimane, B.B. (2011). Molecular characterization of *Fasciola* spp. From the endemic area of northern Iran based on nuclear ribosomal DNA sequences. *Exp. Parasitol.*, 128(3): 196 – 204. DOI: 10.1016/j.exppara.2011.03.011

Anonymous (1995). Control of foodborne trematode infections. Report of a WHO Study Group, World Health Organ. *Tech. Rep. Ser.,* 849: 1 – 157

Anuracpreeda, P., Wanichanon, C., Sobhon, P. (2009). *Fasciola gigantica*: immunolocalization of 28.5 kDa antigen in the tegument of metacercaria and juvenile fluke. *Exp. Parasitol.*, 122: 75 – 83. DOI: 10.1016/j.exppara.2009.03.007

Ashrafi, K., Valero, M.A., Panova, M., Massoud, J., Mas-Coma, S. (2006). Phenotypic analysis of adults of *Fasciola hepatica, Fasciola gigantica* and intermediate forms from the endemic region of Gilan, Iran. *Parasitol. Int.*, 55: 249 – 260. DOI: 10.1016/j.parint.2006.06.003

Attallah, A.M., Karawia, E.A., Ismail, H., Tabll, A.A., Nawar, A.A., Ragab, W.A., Abdel Aziz, M.M., El-Dosoky, I. (2002). Identification and characterization of a 26 – 28 kDa circulating antigen of *Fasciola gigantica*. *Ann. Trop. Med. Parasitol.*, 96(3): 271 – 282. DOI: 10.1179/000349802125000754

Bradford, M.M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the

Principle of Dye Binding. *Anal. Biochem.,* 72: 248 – 254. DOI: 10.1016/0003-2697(76)90527-3

Cervi, L.A., Rubinstein, H., Masih, D.T. (1992). Serological, electrophoretic and biological properties of *Fasciola hepatica* antigens. *Rev. Inst. Med. Trop. Sao Paulo*, 34: 517 – 525. DOI: 10.1590/S0036-46651992000600005

Charlier, J., Meulemeester, L., Claerebout, E., Williams, D., Vercruysse, J. (2008). Qualitative and quantitative evaluation of coprological and serological techniques for the diagnosis of fascioliasis in cattle. *Vet. Parasitol.,* 153: 44 – 51. DOI: 10.1016/j.vetpar.2008.01.035

Choe, S., Nguyen, T., Kang, T., Kweon, C., Kang S. (2011). Genetic analysis of *Fasciola* isolates from cattle in Korea based on second internal transcribed spacer (ITS-2) sequence of nuclear ribosomal DNA. *Parasitol. Res.*, 109: 833 – 839. DOI: 10.1007/s00436-011-2323-6

Coles, G.C., Rubano, D. (1988). Antigenicity of a proteolytic enzyme of *Fasciola hepatica. J. Helminthol.*, 62: 257 – 260. DOI: 10.1017/S0022149X00011627

Cornelissen, J.B.W.J., Gaasenbeek, C.P.H., Boersma, W., Borgsteede, F.H.M., Van Milligen, F.J. (1999). Use of pre-selected epitope cathepsin–L1 in a highly specific peptide-based immunoassay for the diagnosis of *Fasciola hepatica* infection in cattle. *Int J Parasitol.,* 29: 685 – 696. DOI: 10.1016/S0020-7519(99)00017-X

Dalton, J., Heffernan, M. (1989). Thiol proteases released in vitroby *Fasciola hepatica. Mol. Biochem. Parasitol.,* 35: 161 – 166. DOI: 10.1016/0166-6851(89)90118-7

Dalton, J.P., McGonigle, S., Rolph, T.P., Andrews, S.J. (1996). Induction of protective immunity in cattle against infection with *Fasciola hepatica* by vaccination with cathepsin L proteinases and with hemoglobin. *Infect. Immun*., 64: 5066 – 5074

De Vera, M.E., Sato, K., Oyong, G., Claveria, F.G. (2009). Comparison of protein profile of co-existing *Fasciola hepatica* and *Fasciola gigantica* parasite in *Bos taurus* (cattle) and *Bubalus bubalis* (Philippine water buffalo). *J. Protozool. Res*., 19: 1 – 9

Dixit, A.K., Dixit, P., Sharma, R.L. (2008). Immunodiagnostic/protective role of cathepsin L cysteine proteinases secreted by *Fasciola* species. *Vet. Parasitol*., 154: 177 – 184. DOI: 10.1016/j.vetpar.2008.03.017

Dorchies, P. (2007). Comparison of methods for the veterinary diagnosis of liver flukes (*Fasciola hepatica*) in cattle, *Bulletin USAMV-CN*, 64(1 – 2): 14 – 19. DOI: 10.15835/buasvmcn-vm:64:1-2:2270

Dowd, A.J., Smith, A.M., McGonigle, S., Dalton, J.P. (1994). Purification and characterisation of a second cathepsin L proteinase secreted by the parasitic trematode *Fasciola hepatica*. *Eur. J. Biochem*., 223: 91 – 98. DOI: 10.1111/j.1432-1033.1994.tb18969.x

Galavani, H., Gholizadeh, S. Tappeh, K.H. (2016). Genetic characterization of *Fasciola* isolates from west Azerbaijan province Iran based on ITS1 and ITS2 sequence of ribosomal DNA. *Iran J. Parasitol*., 11: 52 – 64. DOI: 10.3923/jp.2016.61.66

Gonenc, B., Sarimehmetolu, H.O., Koro, M., Kiracali, F. (2004). Comparison of crude and excretory-secretory antigens for the diagnosis of *Fasciola hepatica* in sheep by western blotting. *Turk. J. Vet. Anim. Sci.,* 28: 943 – 949

Goreish, I.A., Williams, D.J., McGarry, J., Abdel Salam, E.B., Majid, A.M., Mukhtar, M.M. (2008). Protein Profile of *Fasciola gigantica* Antigens. *Sudan J. Vet. Res*., 23: 1 – 9

Gorman, T., Aballay, J., Fredes, F., Silva, M., Aguillon, J.C., Alcaino, H.A. (1997). Immunodiagnosis of fasciolosis in horses and pigs using western blots. *Int. J. Parasitol.,* 27: 1429 – 1432. DOI: 10.1016/S0020-7519(97)00090-8

Gupta, S.C., Ghosh, S., Joseph, D., Singh, B.P. (2003). Diagnosis of experimental *Fasciola gigantica* in cattle by affinity purified antigen. *Indian J. Anim. Sci.,* 7: 963 – 966

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.,* 41: 95 – 98

Hammami, H., Ayadi, A., Camus, D., Dutoit, E. (1997). Diagnostic value of the demonstration of specific antigen of *Fasciola hepatica* by western blot technique. *Parasite,* 4: 291 – 295. DOI: 10.1051/parasite/1997043291

Hashimoto, K., Watanabe, T., Liu, C.X., Init, I., Blair, D., Ohnishi, S. (1997). Mitochondrial DNA and nuclear DNA indicate that the Japanese *Fasciola* species is *F. gigantica*. *Parasitol. Res.,* 8: 220 – 225

Hassan, S.E., Ghazy, A.A., Abdel-Rahman, E. (2014). Value of serum and milk ELISAs in lactating buffaloes using *Fasciola gigantica* partially purified somatic antigen. *Glob. Vet.*, 13(3): 365 – 372. DOI: 10.5829/idosi.gv.2014.13.03.84270

Hillyer, G.V. (1985). Induction of immunity in mice to *Fasciola hepatica* with a *Fasciola/Schistosoma* cross-reactive defined immunity antigen. *Am. J. Trop. Med. Hyg.*, 34: 1127 – 1131. DOI: 10.4269/ajtmh.1985.34.1127

Hillyer, G.V., Soler de Galanes, M., Battisti, G. (1992). *Fasciola hepatica*: host responders and non responders to parasite glutathione S-transferase. *Exp. Parasitol*., 75(2): 176 – 186. DOI: 10.1016/0014-4894(92)90177-C

Huang, W.Y., He B., Wang, C.R., Zhu, X.Q. (2004). Characterization of *Fasciola* species from Mainland China by ITS-2 ribosomal DNA sequence. *Vet. Parasitol.,* 120: 75 – 83. DOI: 10.1016/j.vetpar.2003.12.006

Ichikawa, M., Itagaki, T. (2010). Discrimination of the ITS1 types of *Fasciola* spp. based on a PCR-RFLP method. *Parasitol. Res.,* 106: 757 – 761. DOI: 10.1007/s00436-010-1724-2

Intapan, P.M., Maleewong, W., Wongkham, C., Tomanakarn, K., Ieamviteevanich, K., Pipitgool, V., Sukolapong, V. (1998). Excretory-secretory antigenic components of adult *Fasciola gigantica* recognized by infected human sera. *Southeast Asian J. Trop. Med. Public Health*, 29: 579 – 583

Intapan, P.M., Maleewong, W., Nateeworanart, S., Wongkham, C., Pipitgool, V., Sukolapong, V., Sangmaneedet, S. (2003). Immunodiagnosis of human fasciolosis using an antigen of *Fasciola gigantica* adult worm with the molecular mass of 27 kDa by a dot ELISA.

Southeast Asian J. Trop. Public Health, 34(4): 713 – 717

Itagaki, T., Tsutsumi, K. (1998). Triploid form of Fasciola in Japan: Genetic relationships between *Fasciola hepatica* and *Fasciola gigantica* determined by ITS-2 sequence of the nuclear rDNA. *Int. J. Parasitol.,* 28: 777 – 781. DOI: 10.1016/S0020-7519(98)00037-X

Itagaki, T., Kikawa, M., Sakaguchi, K., Shimo, J., Terasaki, K., Shibahara, T. (2005a). Genetic characterization of parthenogenetic *Fasciola* sp. in Japan on the basis of the sequences of ribosomal and mitochondrial DNA. *Parasitology*, 131: 679 – 685. DOI: 10.1017/S0031182005008292

Itagaki, T., Kikawa, M., Terasaki, K., Shibahara, T., Fukuda, K. (2005b). Molecular characterization of parthenogenic *Fasciola* sp. in Korea on the basis of DNA sequence of ribosomal ITS1 and mitochondrial NDI gene. *J. Vet. Med. Sci.*, 67: 1115 – 1118. DOI: 10.1292/jvms.67.1115

Kamel, H.H., Saad, G.A., Sarhan, R.M. (2013). Dot-blot immunoassay of *Fasciola gigantic*a infection using 27 kDa and adult worm regurge antigens in Egyptian patients. *Korean J. Parasitol*., 51: 177 – 182. DOI: 10.3347/kjp.2013.51.2.177.

Karimi, A. (2008). Genetic diagnosis of Fasciola species based on 18S ribosomal DNA sequences. *J. Biol. Sci.,* 7: 1166 – 1173. DOI: 10.3923/jbs.2008.1166.1173

Khabisi, S.A., Sarkari, B. (2016). Detection of *Fasciola hepatica* and *Fasciola gigantica* commonand uncommon antigens, using rabbit hyper immune serum raised against their excretory-secretory and somatic antigens. *J. Parasit. Dis.,* 40(4): 1552 – 1557. DOI: 10.1007/s12639-015-0726-5

Khan, M.A.H., Shareef, P.A.A., Rehman, A., Ullah, R., Rehman, L., Abidi, S.M.A. (2017). Genotoxic potential of *Fasciola gigantica* infection in experimentally infected rabbits. *J. Parasit. Dis.,* 41: 423 – 428. DOI: 10.1007/s12639-016-0820-3

Kumar, S., Stecher, G., Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.*, 33: 1870 – 1874. DOI: 10.1093/molbev/msw054

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680 – 685. DOI: 10.1038/227680a0

Latchumikanthan, A., Soundararajan, C., Dhinakarraj, G., Basith, S.A. (2012). Purification and Characterization of Excretory/Secretory Antigens of *Fasciola gigantica. J. Vet. Anim. Sci.*, 8(1): 14 – 18

Le, T.H., De, N.V., Agatsuma, T., Nguyen, T.G.T., Nguyen, Q.D., McManus, D.P. (2008). Human fascioliasis and the presence of hybrid/introgressed forms of *Fasciola hepatica* and *Fasciola gigantica* in Vietnam. *Int. J. Parasitol.,* 38: 725 – 730. DOI: 10.1016/j.ijpara.2007.10.003

Maleewong, W., Intapan, P.M., Tomanakan, K., Wongkham, C. (1997). Antigenic components of somatic extract from adult *Fasciola gigantica* recognized by infected human sera. *Asian Pac. J. Allergy Immunol.*, 15: 213 – 218

Maleewong, W., Wongkhan, C., Intapan, P.M., Pipitgool, V. (1999). *Fasciola gigantica* specific antigens: purification by a continuous-elusion method and its evaluation for the diagnosis of human Fascioliasis. *Am. J. Trop. Med. Hyg*., 61: 648 – 651

Marcilla, A., Bargues, M.D, Mas-Coma, S. (2002). A PCR-RFLP assay for the distinction between *Fasciola hepatica* and *Fasciola gigantica*. *Mol. Cell Probes*, 16: 327 – 343. DOI: 10.1006/mcpr.2002.0429

Meshgi, B., Eslami, A., Hemmatzadeh, F. (2008). Determination of somatic and excretory antigens of *Fasciola hepatica* and *Fasciola gigantica* using SDS-PAG. Iranian *J. Vet. Res*., 9: 77 – 80. DOI: 10.22099/IJVR.2008.526

Moghaddam, A.S., Massoud, J., Mahmoodi, M., Mahvi, A.H., Periago, M.V., Artigas, P., Fuentes, M.V., Bargues, M.D., Mas-Coma, S. (2004). Human and animal fascioliasis in Mazandaran province, northern Iran. *Parasitol. Res.,* 94(1): 61 – 69. DOI: 10.1007/s00436-004-1169-6

Moxon, J.V., LaCourse, E.J., Wright, H.A., Perally, S., Prescott, M.C., Gillard, L.J., Barrett, J., Hamilton, J.V., Brophy, P.M. (2010). Proteomic analysis of embryonic *Fasciola hepatica*: characterization and antigenic potential of a developmentally regulated heat shock protein. *Vet. Parasitol*., 169: 62 – 75. DOI: 10.1016/j.vetpar.2009.12.031

Nour Eldin, M.S., EL- Ganaini, G.A., Abou EL-Enin, A.M., Hussein, E.M., Sultan, D.M. (2004). Evaluation of seven assays detecting serum immunoglobulin classes and subclasses and salivary and faecal secretory IgG against *Fasciola* excretory/secretory (ES) antigen in diagnosing Fascioliasis. *J. Egypt. Soc. Parasitol*., 34: 691 – 704

Olaechea, F.V., Christensen, N.O., Henriksen, S.A. (1990). A comparison of filtration, concentration and thick smear technique in the diagnosis of *Schistosoma* boois infection in cattle and goats. *Acta Trop.,* 47: 217 – 221

Parkhouse, R.M., Almond, N.M., Cabrera, Z., Harnett, W. (1987). Nematode antigens in protection, diagnosis and pathology. *Vet. Immunol. Immunopath*., 17: 313 – 324. DOI: 10.1016/0165-2427(87)90150-4

Periago, M.V., Artigas, P., Khoubbane, M., Moghaddam, A.S., Ashrafi, K., Mansoorian, A.B., Masoud, J., Bargues, Mas-Coma, S. (2004). Genotypic analysis of adult liver flukes from Iran based on the ribosomal M.D. DNA markers ITS-1 and ITS-2. In: Symposium on schistosomiasis and distomatoses. Multidisciplinarity for parasites, vectors and parasitic diseases (eds. B. Mas-Coma, M.D. Bargues, M.A. Valero, V. Esteban and H.K.E. Mott), *IX European Multicolloquium of Parasitology,* Valencia, Spain. 286 – 287

Peters, P.A., El Alamy, M., Warren, K.S., Mahmoud, A.A. (1980). Quick Kato smear for field quantification of *Schistosoma* mansoni eggs. *Am J Trop Med Hyg.,* 29: 217 – 219. DOI: 10.4269/ajtmh.1980.29.217

Phiri, I.K., Phiri, A.M., Harrison, L.J.S. (2006). Serum antibody isotype responses of Fasciola-infected sheep and cattle to excretory and secretory products of Fasciola species. *Vet. Parasitol.,* 141: 234 – 242. DOI: 10.1016/j.vetpar.2006.05.019

Prasad, P.K., Tandon, V., Biswal, D.K., Goswami, L.M., Chatterjee, A. (2008). Molecular identification of the Indian liver fluke, *Fasci-*

ola (Trematoda: fasciolidae) based on the ribosomal internal transcribed spacer regions. *Parasitol. Res*., 103: 1247 – 1255

Raina, O.K., Jacob, S.S., Sanker, M., Bhattacharya, D., Bandyopadyay, S., Varghese, A., Chamuah, K., Lalrinkima, H. (2015). Genetic characterization of *Fasciola gigantica* from different geographical regions of India by ribosomal DNA markers. *J. Parasit. Dis*., 39(1): 27 – 32. DOI: 10.1007/s12639-013-0276-7

Roeber, F., Jex, A.R., Gasser, R.B. (2013). Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance – an Australian perspective. *Parasit. Vectors*, 6: 153. DOI: 10.1186/1756-3305-6-153

Rokni, M.B., Baghernejad, A., Mohebali, M., Kia, E.B. (2004). Enzyme linked immunotransfer blot analysis of somatic and excretory-secretory antigens of *Fasciola hepatica* in diagnosis of human Fascioliasis. *Iranian J. Public Health*, 33: 8 – 13

Rzhetsky, A. and Nei, M. (1992). A simple method for estimating and testing minimum evolution trees. *Mol. Biol. Evol.*, 9: 945 – 967. DOI: 10.1093/oxfordjournals.molbev.a040454

Sampaio-Silva, M.L., Da Costa, J.M., Da Costa, A.M., Pires, M.A., Lopes, S.A., Castro, A.M., Monjour, L. (1996). Antigenic components of excretory–secretory products of adult *Fasciola hepatica* recognized in human infections. *Am. J. Trop. Med. Hyg.*, 54: 146 – 148. DOI: 10.4269/ajtmh.1996.54.146

Santiago, N., Hillyer, G.V., Garcia-Rosa, M., Morales, M.H. (1986). Identification of functional *Fasciola hepatica* antigens in experimental infections in rabbits. *Am. J. Trop. Med. Hyg.*, 35: 135 – 140. DOI: 10.4269/ajtmh.1986.35.135

Santiago, N., Hillyer, G.V. (1988). Antibody profiles by EITB and ELISA of cattle and sheep infected with *Fasciola hepatica*. *J. Parasitol*., 74: 810 – 818. DOI: 10.2307/3282259

Shafiei, R., Sarkari, B., Sadjjadi, S.M., Mowlavi, G.R., Moshfe, A. (2014). Molecular and morphological characterization of *Fasciola* spp. isolated from different host species in a newly emerging focus of human fascioliasis in Iran. *Vet. Med. Int.,* 405740. DOI: 10.1155/2014/405740

Sharma, R.L., Dhar, D.N., Raina, O.K. (1989). Studies on the prevalence and laboratory transmission of fascioliasis in animals in the Kashmir valley. *Br. Vet. J.*, 145: 57

Shoriki, T., Ichikawa-Seki, M., Suganuma, K., Naito, I., Hayashi, K., Nakao, M., Aita, J., Mohnta, U,K., Inoue, N., Murakami, K., Itagaki, T. (2015). Novel methods for the molecular discrimination of *Fasciola* spp. on the basis of nuclear protein coding genes. *Parasitol. Int.,* 65: 180 – 183

Smith, A.M., Dowd, A.J., McGonigle, S., Keegan, P.S., Brennan, G., Trudgett, A., Dalton, J.P. (1993). Purification of a cathepsin L-like proteinase secreted by adult *Fasciola hepatica*. *Mol. Biochem. Parasitol*., 62: 1 – 8. DOI: 10.1016/0166-6851(93)90171-S

Sobhon, P., Anantavara, S., Dangprasert, T. (1996). *Fasciola gigantica*: Identification of adult antigens, their tissue sources and possible origins. *J. Sci. Soc. Thai.,* 22: 143 – 162

Soulsby, E.J.L. (1982). Helminths, arthropods and protozoa of domesticated animals. seventh ed. Bailliere Tindall, London Great Britain, 64 – 65. DOI: 10.1016/0035-9203(84)90110-X

Thienpont, D., Rochetle, F., Vanparijis, O.F.J. (1979). Sedimentation. In: Diagnosing Helminthiasis through Coprological Examination. *Janssen Research Foundation*, Beerse, Belgium, 34 – 36

Thompson, J.D., Higgins, D.G., Gibson, T.J. (1994). CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*., 22: 4673 – 4680

Toledo, R., Bernal, M.D., Marcilla, A. (2011). Proteomics of foodborne trematodes, *J. Proteomics,* 74: 1485 – 1503. DOI: 10.1016/j.jprot.2011.03.029

Upadhyay, A.K., Kumar, M. (2002). SDS-PAGE analysis of *Fasciola gigantica* antigen. *J. Immunol. Immunopathol.*, 4: 91 – 92

Vercruysse, J., Claerebout, E. (2001): Anthelmintic treatment vs. non treatment in cattle: defining the threshold. *Vet. Parasitol*., 98: 195 – 214. DOI: 10.1016/S0304-4017(01)00431-9

Yokananth, S., Ghosh, S., Gupta, S.C., Suresh, M.G., Saravanan, D. (2005). Characterization of specific and cross-reacting antigens of *Fasciola gigantica* by Immunoblotting. *Parasitol. Res*., 7(1): 41 – 48. DOI: 10.1007/S00436-005-1371-1

DOI 10.2478/helm-2019-0007

HELMINTHOLOGIA, 56, 2: 108 – 117, 2019

Intestinal parasites of pets and other house-kept animals in Moscow

O. P. KURNOSOVA, M. V. ARISOV*, I. M. ODOYEVSKAYA

All-Russian Scientific Research Institute of Fundamental and Applied Parasitology of Animals and Plants named after K.I. Skryabin - Branch of the Federal State Budget Scientific Institution «Federal Scientific Center - All- Russian Scientific Research Institute of Experimental Veterinary Medicine named after K.I. Skryabin and Y.R. Kovalenko the Russian Academy of Sciences», 28, Bolshaya Cheremushkinskaya street, Moscow, Russia, 117218, *E-mail: *director@vniigis.ru*

Article info

Received August 23, 2018
Accepted March 1, 2019

Summary

A study screening pet animals (dogs, cats, chinchillas, ferrets, guinea pigs, rabbits, primates, reptiles, and hedgehogs) within Moscow city limits for intestinal parasitic diseases has been conducted over a period of 6 years. According to the study, parasitic infections caused by intestinal protozoa are found in pet animals more frequently than by intestinal helminths. Although dogs and cats exhibit the highest level of diversity of intestinal parasite species, in the group of exotic animals, helminth infection are found much less frequently and parasitic fauna is represented mostly by intestinal protozoa with a high percentage of mixed infection. The most widespread helminth infection of dogs and cats is toxocarosis (respectively 2.5 and 5.7 %) and the most widespread protozoan infection is *Giardia* sp. (9.8 and 4.6 %). *Giardia* sp. was found in 47.4 % of chinchillas, *Cryptosporidium* sp. was more frequently found in ferrets (6.55 %), protozoa from the family Trichomonadida was found in guinea pigs (9 %), *Eimeria* sp. in rabbits (13.9 %), *Acanthocephal*a in primates (15.7 %), and eggs from the genera*Oxyurida* (59 %), along with protozoa from the family Trichomonadida, in reptiles. *Capillaria* sp. was most prevalent in hedgehogs (33.4 %). *Acanthocephala* eggs, as well as protozoa from the *Giardia* and *Entamoeba* genera, were more frequently found in primates. Parasites common to animals and humans, which may become a source of infection for the latter under certain conditions, have been identified in pet animals.

Keywords: pets; intestinal parasites; helminths; protozoa; prevalence of infection, Moscow; Russia

Introduction

Moscow is home to a huge number of pet animals, and most of them are cats and dogs. Ferrets, guinea pigs, chinchillas, and rabbits are also often kept in urban apartments. The number of owners who keep hedgehogs, rats, reptiles, and birds is smaller. Small animal enclosures in large shopping malls that often hold squirrels and primates have recently become very popular. Also popular are petting zoos with guinea pigs, rabbits, and mini pigs. Animals have become integral to the life of people living in major metropolitan areas. In most cases, they are full-fledged family members.

Constant veterinary supervision of animal health status, various kinds of preventative activities conducted on a constant basis, the owner's knowledge of animal diseases and their regular visiting city's veterinary clinics contribute to the reduction of some infectious diseases of the animals in the city and improve their quality of life in the event of chronic conditions. Without a doubt, however, certain diseases continue to be a matter of urgency despite the preventative measures that are being taken in a regular manner. Intestinal parasitic diseases caused by various species of helminths and protozoa represent a special case among these pet animal diseases. For this group of diseases, urban areas are an unique

* – corresponding author

Table 1. Indices of infection prevalence (IP) for parasitic diseases in pet dogs.

Types of Parasites Indices of Infection Prevalence	Indices of Infection Prevalence						Total infected (samples), (IP, %)
	2012 (IP, %)	2013 (IP, %)	2014 (IP, %)	2015 (IP, %)	2016 (IP, %)	2017 (IP, %)	
Helminths							
T. canis	12 (4)	5 (1.8)	6 (2.3)	3 (0.98)	6 (2.3)	11 (2.7)	43 (2.4)
T. leonina	3 (1)	2 (0.75)	1 (0.38)	-	-	2 (0.49)	8 (0.45)
Ancylostomidae	2 (0.67)	1 (0.37)	1 (0.38)	4 (1.3)	1 (0.38)	1 (0.24)	10 (0.57)
Ancylostoma caninum	-	-	-	-	1 (0.38)	-	1 (0.05)
T. vulpis	-	3 (1.1)	1 (0.38)	1 (0.32)	-	-	5 (0.28)
Larvae Strongyloides sp.	1 (0.3)	-	1 (0.38)	1 (0.32)	3 (1.1)	10 (2.4)	16 (0.91)
Capillaria sp.	1 (0.3)	1 (0.37)	-	1 (0.32)	-	-	3 (0.17)
Taenia sp.	-	-	3 (1.1)	-	1 (0.38)	-	4 (0.22)
S. lupi	-	1 (0.37)	-	-	-	-	1 (0.05)
Examined samples, total	295	266	259	305	260	403	1752
Samples infected with helminths	19	12	12	10	11	24	88
Prevalence of infection (%)	6.4	4.5	4.6	3.2	4.2	5.9	5
Protozoa							
Giardia sp.	23 (7.7)	21 (7.8)	24 (9.2)	23 (7.5)	21 (8)	60 (14.8)	172 (9.8)
Sarcocystis sp.	20 (6.7)	28 (10.8)	21 (8.1)	6 (1.9)	14 (5.3)	21 (5.2)	110 (6.2)
Cystoisospora sp.	7 (2.3)	6 (2.2)	9 (3.4)	1 (0.3)	4 (1.5)	9 (2.2)	36 (2)
C.canis	4 (1.3)	1 (0.37)	3 (1.1)	4 (1.3)	2 (0.76)	3 (0.7)	17 (0.97)
Cryptosporidium sp.	1 (0.33)	7 (2.6)	4 (1.5)	2 (0.6)	-	4 (0.24)	18 (1)
N. caninum or H. heydorni	-	2 (0.75)	2 (0.77)	1 (0.3)	1 (0.38)	1 (0.24)	6 (0.34)
Trichomonadidae	1 (0.33)	-	-	-	-	3 (0.7)	4 (0.22)
Samples infected with protozoa	56	65	63	37	42	101	364
Prevalence of infection (%)	18.9	24.4	24.3	12.1	16.1	25	20.7

environment that creates preconditions for circulating pet animal's infections due to widespread importation of animals from other countries and introduction of previously unseen parasite types (Kurnosova, 2009). The high concentration of pet dogs, presence of stray animals, use of common walking areas, and inadequate feces collection within city limits tend to maintain and transmit the source of infection to other animals (Erofeeva & Doronina, 2017). Many parasitic diseases, especially helminthiases, are low-key infections that are subclinical. In such cases, pet animals become a source of infection for an extended period of time. The course and duration of the desease depends on many factors, such as the type and intensity level of the infestation, the age and immune system status of the animal, and the presence of any concomitant underlying diseases. In general, intestinal parasitic diseases can seriously harm the health of the animal (Kurnosova, 2009; Panova, 2011; Stepanov, 2014).

There are parasitic diseases common to animals and humans that pose a serious threat to the latter (toxocarosis, alveolar echinococcosis, cystic echinococcosis, and toxoplasmosis) (Malysheva *et al*., 2013; Shcheveleva *et al*., 2016). Due to the biological features of some parasitic diseases, the risk of direct infection from infected animal to human is very low, because some helminth or protozoan

Fig.1. Eggs of *Toxocara canis* in dog.

Table 2. Indices infection of prevalence (IP) for parasitic diseases in pet cats.

Species of Parasites	Indices of Infection Prevalence						Total infected(samples), (IP, %)
	2012 (IP, %)	2013 (IP, %)	2014 (IP, %)	2015 (IP, %)	2016 (IP, %)	2017 (IP, %)	
Helminths							
T. cati	13 (6.2)	8 (4.2)	11 (5.9)	9 (4.3)	11 (5.8)	21 (7.3)	73 (5.7)
T. leonina	1 (0.47)	-	-	-	-	-	1 (0.07)
D. caninum	3 (1.4)	5 (2.6)	4 (2.1)	2 (0.96)	1 (0.5)	1 (0.35)	16 (1.2)
Taenia sp.	-	3 (1.5)	-	4 (1.9)	1 (0.5)	2 (0.7)	10 (0.79)
Capillaria sp.	-	1 (0.5)	-	1 (0.48)	4 (2.1)	1 (0.35)	7 (0.5)
Ancylostomatidae	-	2 (1)	-	-	-	-	2 (0.15)
Examined samples, total	209	188	184	207	189	284	1261
Samples infected with helminths	17	18	15	16	17	25	109
Prevalence of infection (%)	1.34	1.4	1.18	1.26	1.34	1.9	8.6
Protozoa							
Giardia sp.	8 (3.8)	7 (3.7)	7 (3.8)	12 (5.7)	11 (5.8)	14 (4.9)	59 (4.6)
Sarcocystis sp.	1 (0.47)	-	5 (2.7)	1 (0.48)	1 (0.52)	1 (0.35)	9 (0.71)
C. rivolta	2 (0.95)	1 (0.5)	2 (1)	1 (2.8)	2 (1)	3 (1)	11 (0.87)
C. felis	5 (2.3)	6 (3.1)	4 (2.1)	6 (0.48)	8 (4.2)	3 (1)	32 (2.5)
Cryptosporidium sp.	4 (1.9)	4 (2.1)	1 (0.5)	5 (2.4)	1 (0.52)	-	15 (1.1)
Trichomonadidae	3 (1.4)	2 (1)	7 (3.8)	2 (0.96)	8 (4.2)	8 (2.8)	30 (2.3)
Samples infected with protozoa	23	20	26	27	31	29	156
Prevalence of infection (%)	1.8	1.58	2	2.1	2.4	2.2	12.3

types need a certain environment residence time, temperature, and humidity to be able to infect. There is, however, a group of so-called “contact” parasitic diseases whose infecting principle is introduced into the environment while already primed for infestation and can be transmitted to humans under certain conditions (dwarf tapeworms, enterobiasis, giardiosis, cryptosporidiosis,

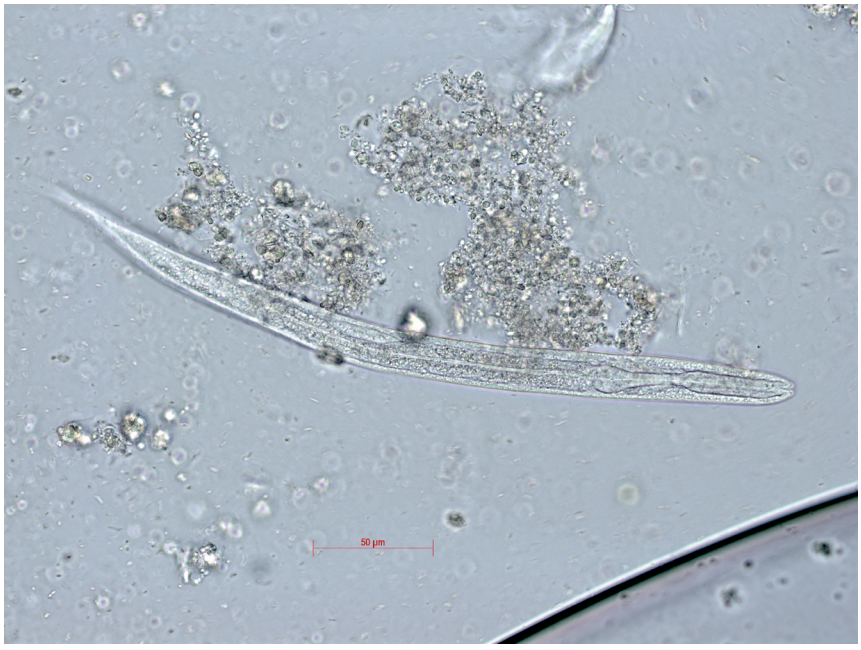

Fig.2. Larva of *Strongyloides* sp. in dog, stage L1.

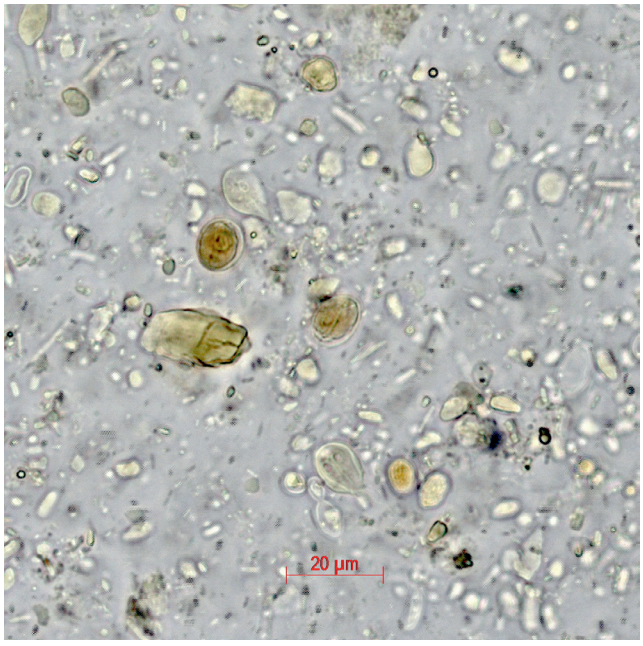

Fig.3. Trophozoites and cysts of *Giardia* sp. in cat.

blastocystosis, many species of amoebas) (Sergiev *et al*., 2006). If personal hygiene is inadequate, shelter staff, pet owners, and young children are at risk.

Many major cities are regularly screening pet animals and stray animals for parasitic diseases: St.Petersburg (Prozorov, 1999); Novosibirsk (Zubareva, 2001, Konyaev & Borcova, 2015); Kostroma (Mikhina, 2008); Moscow (Kurnosova, 2009; Panova, 2011; Uspensky *et al*., 2011; Loshchinin *et al*., 2015; Kurnosova & Odoyevskaya, 2017); London (Upjonh & Cobb, 2010); Italy, Netherlands, United Kingdon (Feng & Xiao, 2011), Calgary (Joffe & Van Niekerk, 2011); Austria, France, Italy, Portugal (Beugnet & Bourdeau, 2014; Banerh & Thamsbord, 2016; Pereira & Martins, 2016). Studies of this type are of particular value if the duration of observation is sufficiently long. In addition to identifying the species composition of the pet animal parasites circulating throughout the city, such a study makes it possible to register new species, take timely remedial and preventative measures, and predict new developments related to the most widespread infections. This publication provides observational data obtained in a 6-year study of disease prevalence in pets in the City of Moscow.

Materials and Methods

The study was conducted at the Veterinary Laboratory "Pasteur". Animal fecal samples were delivered to the laboratory in special plastic containers or glassware.

Feces obtained from various animal species were studied using a variety of methods including flotation with sodium chloride and ammonium nitrate solutions. Some flotation and sedimentation techniques are used only in Russia. For instance, Kotelnikov-Varenichev's and Kotelnikov-Chrenov's methods are centrifugal flotation techniques, which have high sensitivity for many helminth species (Kotelnikov, 1974; Kotelnikov & Chrenov 1980), ether and formalin precipitation, and direct smear microscopy (MUC 4.2.3145-13). Sometimes all the research methods were used simultaneously based on the particular features of the approach used to identify various species of protozoa, in accordance with the nature of the fecal sample under test.

Microscopy was performed at 10x and 40x magnification using a Lomo microscope (Joint stock company Lomo, Russia). The species of helminth eggs and protozoan cysts were determined based on their morphological features and size.

After examination, the material samples were disinfected by autoclaving.

Ethical Approval

The animal study was in compliance with all the 209 relevant national regulations and institutional policies relating to the care and use of animals in Russia.

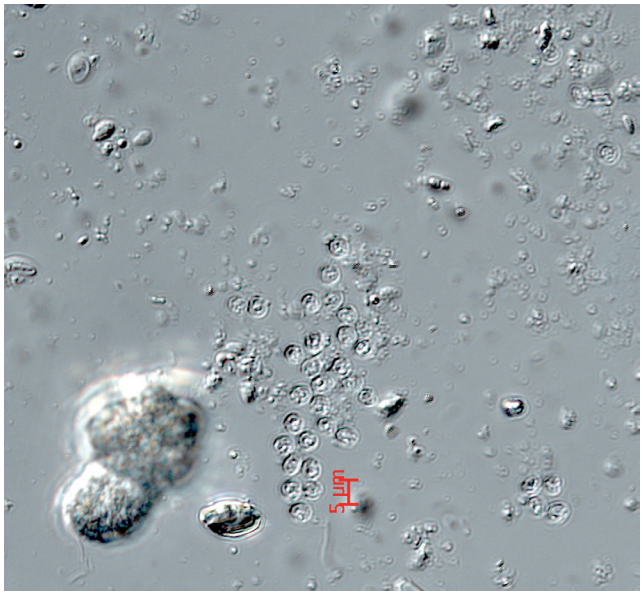

Fig.4.Oocysts of *Cryptosporidium* sp. in ferret.

Results

The pet animal study was conducted for 6 years from 2012 to 2017. During this period, we studied fecal samples from 1752 dogs (*Canis lupus*) , 1261 cats (*Felis catus*), 323 ferrets (*Mustela putorius*), 217 chinchillas (*Chinchilla lanigera*), 132 guinea pigs (*Cavia porallus*), 165 rabbits (*Oryctolagus cuniculus*), 65 tamarins (*Saguinus oedipus*), 50 marmoset (*Callithrix jacchus*), 10 galago (*Galago senegalensis*), 10 chimpanzee (*Pan troglodytes*), 10 turtles (*Testudo horsfieldii*), 11 geckos (*Gekkonidae*), 21 hedgehogs (*Erinaseus europaeus*).

In the dogs, we discovered eight different helminths and seven dif-

ferent protozoa, i.e. *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, Ancylostomatidae , *Ancylostoma caninum*, *Spirocerca lupi*, *Taenia* sp., *Capillaria* sp.; *Sarcocystis* sp., *Cystoisospora canis*, *Cystoisospora* sp., *Giardia* sp., *Neospora caninum* and/or *Hammondia heydorni,* Thrichomonadidae, and *Cryptosporidium* sp. (Table 1).

T.canis eggs were found in 43 samples (2.4 % infection prevalence (IP) is the percentage of a infected animals in the total population) (Fig. 1)., *T.leonina* eggs in 8 samples (0.4 % IP), *Strongyloides* sp. larvae in 16 cases (0.9 % IP) (Fig. 2), *Trichuris vulpis* in 5 samples (0.28 % IP), *S.lupi* in 1 sample (0.05 % IP), *Taenia* sp. in 4 cases (0.2 % IP), Ancylostomatidae in 10 cases (0.5 % IP), *A.caninum* in one case (0.05 % IP), and *Capillaria* sp. in 3 cases (0.17 % IP). Intestinal protozoa including *Giardia* sp. were identified in 172 cases (9.8 % IP), *Sarcocystis* sp. in 110 cases (6.2 % IP), *Cystoisospora* sp. in 36 cases (2 % IP), *C.canis* in 17 cases (0.9 % IP), *Cryptosporidium* sp. in 18 cases (1 % IP), *N.caninum* or/and *H.heydorni* in 6 cases (0.34 % IP), and Thrichomonadidae in 4 cases (0.2 % IP).

In the case of mixed infections are most frequently caused by a

Table 3. Indices of prevalence of infection (IP) by intestinal parasites in other house-kept animals.

Animal species; Type of infestation	Indices of Infection (IP, %)						Total Infected (IP, %)
	2012	2013	2014	2015	2016	2017	
Ferrets							
Capillaria sp.	1 (2.8)	-	-	-	-	-	1 (0.3)
Cryptosporidium sp.	2 (5.7)	7 (14.2)	5 (9.2)	5 (8.6)	1 (1.8)	1 (1.3)	21 (6.5)
Eimeria sp.	1 (2.8)	1 (2)	2 (3.7)	3 (5.1)	8 (15)	2 (2.7)	17 (5.2)
Giardia sp.	-	1 (2)	-	2 (3.4)	-	2 (2.7)	5 (1.5)
Total Examined	35	49	54	58	53	74	323
Of them, infected	4	9	7	10	9	5	41
IP, %	11.4	18.3	12.9	17.2	16.9	6.7	12.6
Chinchillas							
Giardia sp.	6 (33.3)	9 (45)	35 (48.6)	24 (46.1)	9 (36)	20 (50)	103 (47.4)
H.nana	1 (5.5)	-	-	-	-	-	1 (0.46)
Blastocystis sp.	-	-	-	1 (1.9)	-	-	1 (0.46)
Entamoeba sp.	-	-	1 (1.3)	1 (1.9)	-	-	2 (0.92)
Trichomonas sp.	1 (5.5)	2 (10)	-	1 (1.9)	-	-	4 (1.84)
Total Examined	18	20	72	52	25	40	217
Of them, infected	8	11	36	27	9	20	111
IP, %	44.4	55	50	51.9	36	50	51.1
Guinea Pigs							
Trichomonas sp.	1 (4.7)	5 (12.8)	4 (30)	1 (7.1)	-	1 (4)	12 (9)
Giardia sp.	-	1 (2.5)	-	-	1 (5)	3 (12)	5 (3.7)
Entamoeba sp.	-	3 (7.6)	1 (7.6)	1 (7.1)	-	-	5 (3.7)
Total Examined	21	39	13	14	20	25	132
Of them, infected	1	9	5	2	1	4	22
IP, %	4.7	23	38.1	14.1	5	16	16.6
Rabbits							
P.ambiguis	-	-	2 (15.3)	-	2 (3.5)	-	4 (2.4)
Giardia sp.	-	-	-	-	2 (3.5)	1 (1.3)	3 (1.8)
Eimeria sp.	-	-	-	-	14 (24.5)	9 (12.5)	23 (13.9)
Total Examined	5	9	13	9	57	72	165
Of them, infected	0	0	2	0	18	10	30
IP, %	0	0	15.3	0	31.5	13.8	18.1

Primates (tamarins, marmosats, saimiri,galago, capuchins, chimpanzee)							
Acanthocephala	-	13 (27)	5 (55.5)	-	-	4 (7)	22 (15.7)
Streptapharagus sp.	-	2 (4.1)	1 (11.1)	1 (7.1)	-	-	4 (2.8)
Blastocystis sp.	-	2 (4.1)	-	1 (7.1)	-	-	3 (2.1)
Entamoeba sp..	-	4 (8.3)	-	1 (7.1)	-	-	5 (3.5)
Trichomonas sp.	-	1 (2)	-	-	1 (8.3)	2 (3.5)	4 (2.8)
Giardia sp.	-	-	-	-	4 (33.3)	1 (1.7)	5 (3.5)
Total Examined	0	48	9	14	12	57	140
Of them, infected	0	22	6	3	5	7	43
IP, %	0	45.8	66.6	21.4	41.6	12.2	30.7
Reptiles (turtles, geckos)							
Oxyurida	-	-	-	1 (0.48)	1 (0.48)	12 (0.48)	13 (59)
Blastocystis sp.	1	--	-	-	1 (0.48)	2 (0.48)	4 (18.1)
Entamoeba sp.	1 (0.48)	-	-	-	-	1 (0.48)	2 (9.5)
Trichomonas sp.	-	-	-	-	1 (0.48)	6 (0.48)	7 (33.3)
Isospora sp.	-	-	-	-	-	3 (0.48)	3 (14.2)
Total Examined	1	0	1	4	0	15	22
Of them, infested	1	0		1	0	15	18
IP, %	100	0	0	25	0	100	81.8
Hedgehogs							
Capillaria sp.	-	-	-	-		7 (0.48)	7 (33.4)
C. striatum				-		2 (0.48)	2 (9.5)
Total Examined	1	-	1	4	0	15	21
Of them, infected	0		0	0	0	9	9
IP, %	0		0	0	0	60	42.8

combination of two species of protozoa, less frequently by a combination of one type of helminths and one type of protozoa. A total of 59 cases of mixed infections in dogs were detected (Table 4).

It was determined that young animals (younger than one year of age) had the highest level of intestinal parasite infection in dogs. Thus, the prevalence of toxocarosis in puppies, based on the total number of positive samples (43 samples were positive, with 31 of them obtained from puppies) was 72 %. The incidence was 62.2 % for *Giardia* sp., 76.4 % for *C. canis*, 69.4 % for *Cystoisospora* sp., and 55.5 % for *Cryptosporidium* sp. The frequency of detection for *Sarcocystis* sp. was 36.3 %.

In the pet cats, we identified the following 6 species of helminths: *T. cati*, *T. leonina*, *Capillaria* sp., *Dipylidium caninum*, Ancylostomatidae , Taenia sp. and 6 species of intestinal protozoa: *Giardia* sp., *Cystoisospora rivolta*, *Cystoisospora felis*, *Sarcocystis* sp., Thrichomonadidae, and *Cryptosporidium* sp.(Table 2)

The most common helminthiasis in cats was toxocarosis, with *T. cati* eggs found in 73 samples (5.7 % infection prevalence (IP)), proglottids of *D.caninum* in 16 samples (1.2 % IP), *Taenia* sp. eggs in 10 samples (0.7 % IP), *Capillaria* sp. in 7 samples (0.5 % IP), *T.leonina* in 1 (0.07 % IP), and Ancylostomatidae in 2 samples (0.15 % IP).

The most common feline protozoan disease was *Giardia* sp., which was found in 59 samples (4.6 % IP) (fig 3). *C. felis* was found in 32 samples (2.5 % IP), *C. rivolta* in 11 samples (0.8 % IP),

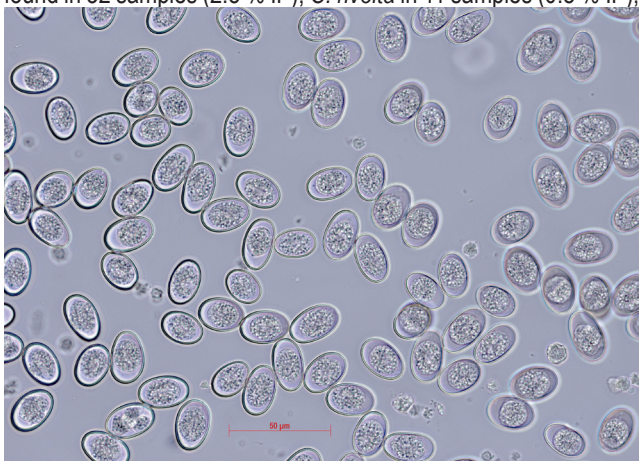

Fig.5. Oocysts of *Eimeria* sp. in ferret.

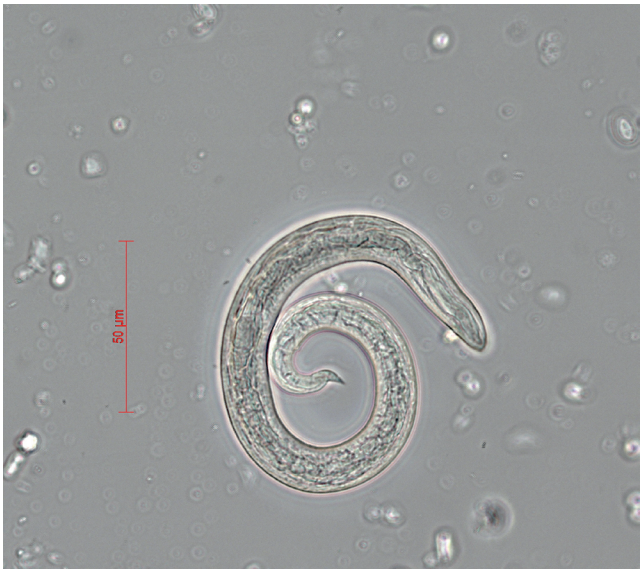

Fig.6. Larva of *Crenosoma striatum* in hedgehog, stage L1.

Sarcocystis sp. in 9 samples (0.7 % IP), *Cryptosporidium* sp. in 15 samples (1.1 % IP), and Thrichomonadidae in 30 samples (2.3 % IP).

There were 26 cases of mixed infections in the cats, most of them were caused by two species of parasites, with only one case involving a combination of 4 species of parasites (Table 4).

In the ferrets, we found a single type of helminths belonging to the genus *Capillaria* (0.3 % infection prevalence (IP) and 3 species of protozoa, i.e. *Cryptosporidium* sp., *Giardia* sp., and *Eimeria* sp. (Table 3). The most frequently seen parasites were *Cryptosporidium* sp., found in 21 cases (6.5 % IP) (Fig. 4), and *Eimeria* sp., found in 17 cases (5.2 % IP) (Fig.5), with *Giardia* sp. cysts represented to a lesser extent. Only one case involving one type of helminths, i.e. *H.nana*, was identified in the chinchillas along with 4 species of protozoa: *Giardia* sp., *Blastocystis* sp., *Entamoeba* sp., and *Trichomonas* sp. The most common intestinal protozoa in the chinchillas were *Giardia* sp. with 193 cases and an IP value of 47.7 %. There were 4 cases of *Trichomonas* sp. (1.8 % IP) and isolated instances of other species (0.4 % IP). The only intestinal parasites found in the guinea pigs were protozoa represented by three species, i.e. *Entamoeba* sp., *Trichomonas* sp., and *Giardia* sp., with the most frequently seen protozoa being *Trichomonas* sp. with 12 cases (9 % IP) and cysts of *Giardia* sp. and amoebas (3.7 % IP). One species of helminths, i.e. *Passalurus ambiguus* with an IP of 2.4 %, 2 species of *Eimeria* sp. with 23 cases (13.9 % IP), and *Giardia* sp. with 3 cases (1.8 % IP), which were encountered much less frequently, were identified in the rabbits. The primates (data for all types) were shown to be 15.8 % infected with Acanthocephala. The stomach nematodes of the *Streptopharagus* sp. are seen much less frequently: 2.8 %. Intestinal protozoa were represented by 4 species: *Giardia* sp. (3.5 % IP), *Blastocystis* sp. (2.1 % IP), *Entamoeba* sp. (3.5 % IP), and *Trichomonas* sp. (2.8 % IP). One species of helminths of the genera *Oxyurida* was identified in four cases in the reptiles along with 4 species of protozoa: *Blastocystis* sp. (18.1 % IP), *Entamoeba* sp. (9.5 % IP), *Trichomonas* sp. (33.3 % IP) and *Isospora* sp. (14.5 % IP). Only two species of helminths were identified in the hedgehogs, i.e. *Capillaria* sp. in 7 cases (33.4 % IP) and *Crenosoma striatum* in 2 cases (9.5 % IP) (Fig. 6).

In certain isolated cases, feces from lemurs (*Lemur catta*) containing helminths of the *Enterobius* sp. (data not listed in the tables) were submitted for examination along with feces from meerkats (*Suricata suricatta*), raccoons (*Procyon lotor*), degu (*Octodon degus*), and echidna (*Tachyglossidae*), in which no intestinal parasites were identified. Protozoa from the *Eimeria* sp., eggs of Catenotaeniidae and eggs of the genera *Oxyurida* were found in squirrels (*Sciurus vulgaris*).

Discussion

The study has shown that *Toxocara* spp. are the most widespread parasites in dogs and cats. Different researchers give different IP values, which may be dependent on the category of the animals under study (examination of all animals in veterinary clinics, animals in shelters, stray animals) (Kurnosova & Odoyevskaya, 2017). It is quite common for *Toxocara* spp. infections to rank first in terms of prevalence in dogs and cats in an urban environment. In addition, cats have a higher level of infection with *Toxocara* sp. than dogs. Currently the role of cats in the epidemiology of human toxocariasis remains unclear and it may be true that the latter are a source of infection for humans to a greater extent than previously thought (Fogt-Wyrwaset *et al*., 2007; Espinoza *et al*., 2010; Panova, 2011). The results of our study are in agreement with other authors (Prozorov, 1999; Zubareva, 2001; Mikhina, 2008; Kurnosova, 2009; Panova, 2011; Kurnosova & Odoyevskaya, 2017;). In this situation, the urban environment favors the spread of toxocarosis infection, in which the main factor of accumulation and spread is the ground of the pet walking areas and the permanent habitat of stray dogs and cats. These conditions ensure constant circulation of this species of helminths, which is fraught with constant re-infection and infection of additional animals. The rest of the helminth species are found less frequently, which is due to the conditions of indirect transmission of a particular species involving intermediate and/or additional hosts. In such a case, cats and dogs get infected when they go away from the city for the summer. In recent years, larvae of *Strongyloides* sp. have been found more frequently than before (Umur & Meral, 2017; Paradies & Iarussi, 2017). This nematode species is found not only in dogs going away from the city or country, but also in animals residing within city limits at all times, mostly in puppies kept in breeding kennels. The Laboratory received material from animals suffering from long-term infection. The course of this type of infection is severe in young animals and disrupts the normal functioning of the gastrointestinal tract, which is manifested in a loss of appetite, copious mucus-laden stools, or intractable diarrheas (Umur &Meral, 2017).

Table 4. Combination mixed infections in dogs and cats.

Mixed infections	Species of animals	Number of combinations
Sarcocystis sp. and *Giardia* sp.	dog	7
Cystoisospora sp. and *Giardia* sp.	dog	6
Cystoisospora sp. and *C. canis*	dog	4
N. caninum and/or *H. heydorni* and *Sarcocystis* sp.	dog	4
T. canis and Giardia sp./ and *Sarcocystis* sp.	dog	3/3
T. canis and *Cystoisospora* sp./ and *Cryptosporidium* sp.	dog	2/2
larvae of *Strongyloides* sp. and C. *canis*/and *Giardia* sp.	dog	2/2
Giardia sp. and *Cryptosporidium* sp./and *C. canis*	dog	2/2
Sarcocystis sp. and *Cystoisospora* sp.	dog	2
larvae of *Strongyloides* sp. and Thrichomonadidae /and *Cystoisospora* sp.	dog	1/1
Ancylostomatidae and *Sarcocystis* sp. /and *Giardia* sp./and C. canis/and *Capillaria* sp.	dog	1/1/1/1
T. canis and *Trichuris vulpis*	dog	1
T. leonina and *Giardia* sp.	dog	1
Giardia sp. and Thrichomonadidae	dog	1
	cat	9
Sarcocystis sp. and *C. canis*	dog	1
Sarcocystis sp. and *Cryptosporidium* sp.	dog	1
T. canis, *C. canis*, and *Cryptosporidium* sp.;	dog	1
T.leonina, *Cryptosporidium* sp., and Thrichomonadidae;	dog	1
Strongyloides sp., *Giardia* sp., and *Cystoisospora* sp.	dog	1
Sarcocystis sp., *Giardia* sp., and *Cryptosporidium* sp.;	dog	1
Sarcocystis sp., *Giardia* sp . and *Cystoisospora* sp.	dog	1
Sarcocystis sp., *N. caninum* and/or *H. heudorni* and *Giardia* sp.	dog	1
T. canis, *T. leonina*, *Giardia* sp., and *C. canis*	dog	1
T. cati and *C. felis*	cat	3
T. cati and *Capillaria* sp./ and *Giardia* sp.	cat	2/1
Giardia sp. and *C. rivolta*	cat	2
T. leonina and *Giardia* sp.	cat	1
Ancylostomatidae and *Cryptosporidium* sp.	cat	1
T. cati and *C. rivolta*/ and *Sarcocystis* sp.	cat	1/1
C. felis and *Cryptosporidium* sp./ and *Giardia* sp./ and *C. rivolta*	cat	1/1/1
Sarcocystis sp. and Thrichomonadidae	cat	1
Ancylostomatidae, *T. cati*, *Taenia* sp. and *Cryptosporidium* sp.	cat	1

Giardiosis ranks first in terms of prevalence among the protozoan diseases of dogs, cats, and chinchillas, with puppies younger than 1 year of age affected most frequently in the case of dogs (Upjonh & Cobb, 2010; Kurnosova, 2014). At the same time, in cats and chinchillas this species of protozoa is found with equal frequency in young and adult animals. Such prevalence of *Giardia* spp. is related to their environmental resistance (Adam, 2001). In dogs, *Giardia* spp. are found more frequently than in cats, which may be due to the animal's daily walking routines and their infection from the environment, as well as to the habit of some dogs to eat the feces of other animals. An animal with weak immunity may become a long-term host to these protozoa. As of now, the role of pet animals in the transmission of this infection to humans within city limits has not been established (Feng & Xiao, 2011; Thompson & Monis, 2004; Thompson & Palmer, 2008; Upjonh & Cobb, 2010). Sarcocystidae are found in dogs more frequently than in cats, which is due to the fact that cats are more often fed dry kibble. In general, however, the prevalence of Sarcocystidae infection has declined in recent years and they now rank second after giardiosis. Infections by flagellated protozoa from the Thrichomonadidae family have recently started cropping up in cats of various ages. These protozoa are sufficiently pathogenic and difficult to treat. The species of trichomonads circulating among cats within city limits have not been established. The animals suffered from chronic diarrhea whenever trichomonads were found. In dogs and cats, *Cryptosporidium* sp. and *Cystoisospora* spp. are less prevalent and are found mostly in young animals(Hamnes & Gjerde, 2007; Garanayak & Gupta, 2017). It should be noted that protozoa from the family Sarcocystidae have not been detected in feline fecal samples during the entire study period, which is compatible with the widely expressed view regarding quick and often asymptomatic excretion of cysts into the environment that goes unnoticed by the owners (Greene, 2011).

In general, and as already shown by previously conducted studies, intestinal protozoa in dogs and cats are found more frequently than helminthosis (Kurnosova, 2009).

Of particular interest is research relating to the group of exotic animals, which are not kept in urban apartments as much as other species. Nevertheless, many species of these animals are gaining in popularity. Monkeys, squirrels, and some species of rodents are normally kept in mini-zoos and large shopping malls in Moscow. They are much less frequently kept by individuals. Owners are increasingly starting to keep rabbits and, less frequently, reptiles and hedgehogs in urban apartments. The research has shown that exotic animals are infected mostly by helminths and protozoa found only in these animal species, although some need to be highlighted their importance in Public Health (Hallinger & Taubert, 2018). The primates infected with Acanthocephala. This species of helminths is widespread in animals, which is due mostly to the fact that the animals are fed insects acting as intermediate hosts to these helminths. While the protozoa from the *Entamoeba* sp. that are most frequently found in monkeys, reptiles, and guinea pigs are represented mostly by *Endolimax nana* and *Iodamoeba* sp., it has not been always possible to determine the species of some amoeba cysts. No dysenteric amoeba (*Entamoeba histolytica*) cysts capable of transmission to humans have been identified over the entire study period (Regan & Yon, 2014; Levecke & Dreesen, 2010; Verweij & Brienen, 2003). However, in addition to the species-specific parasites, helminths and protozoa of a zoonotic nature (eggs of *Hymenolepsis nana*, blastocysts) have also been found in this category of animals under study (Cian & Safadi, 2017). Certainly, when an exotic animal is bought, the animal should be screened for parasitic diseases. In addition, animals leaving city or country limits or those participating in hunting, actively catching and eating insects, rodents, and amphibians may get infected by zoonotic diseases (Hallinger & Taubert, 2018). The same danger should be kept in mind when brining the younglings of wild animals from the forest.

Thanks to the veterinary and medical awareness-building work by the mass-media and the Internet, pet owners have become sufficiently knowledgeable about many issues relating to animal diseases and various preventative activities. Nevertheless, studies have shown that under urban conditions parasitic diseases are relevant both to veterinary science and to medicine (Stepanov, 2014; Shcheveleva *et al*., 2016).

Thus, veterinarians and pet owners should take the species of their animal, it's origin, living and feeding conditions into consideration in order to take preventative measures aimed at timely identification of parasitic infections and their correct treatment under veterinary supervision, which will ensure the animal's health and protect the environment from the spread of infections. Studying a wide range of pet animals under urban conditions has great value not only from a practical standpoint, but also in terms of determining the epizootic situation for all the major parasitic diseases in general and clarifying the human risks associated with the zoonotic nature of the parasitic infections.

Conclusion

The study has shown the widespread circulation of intestinal parasitic infections of house-kept animals in the city of Moscow. The high concentration of animals within confined areas promotes constant circulation of numerous helminths and protozoa among pet animals. *Toxocara* spp. and *Giardia* sp.were found more frequently in dogs and cats together with other parasitic agents are a pressing issue both for veterinary science and for medicine. In summary, helminths and protozoa capable of transmission to humans have been identified among a wide range of pet animal infections in an urban environment.

Conflict of Interest

Authors state no conflict of interest.

References

Adam, R.D. (2001): Biology of *Giardia lamblia*. *Clin. Microbiol. Rev*., 14(3): 447 – 475

Erofeeva, V.V., Doronina, G.N. (2017): The threat of spreading helminth eggs in the soil of urban areas. *The Young Scientist*, 22: 222 – 224 (In Russian)

Espinoza, Y.A., Huapaya, P.E., Roldan, W.H. (2010): Seroprevalence of human toxocariasis in Andean communities from the Northeast of Lima. *Rev. Inst. Med. Trop. Sau Paulo*, 52: 31 – 36

Baneth, G., Thamsborg, S.M., Otranto, D.O., Guillot, J., Blaga, R., Deplazes, P., Solano-Gallego, L. (2016): Major Parasitic Zoonoses Associated with Dogs and Cats in Europe. *J. Comp. Pathol*., 155: 54 – 74. DOI: 10.1016/j.jcpa.2015.10.179

Becker, A.C, Kraemer, A, Epe, C., Strube, C. (2016): Sensitivity and efficiency of selected coproscopical methods-sedimentation, combined zinc sulfate sedimentation-flotation, and McMaster method. *Parasitol. Res*., (113): 2401–2406. DOI: 10.1007/s00436-016-5003-8

Beugnet, F., Bourdiau, P., Chalvet-Monfray, R., Cozma, V., Farkas, R., Guillot, J., Halos, L., Joachim, A., Losson, B., Miró, G., Otranto, D., Renaud, V., Rinaldi, L. (2014): Parasites of domestic owned cats in Europe: co-infestations and risk factors. *Parasit. Vectors*, (7): 291. DOI: 10.1186/1756-3305-7-291

Feng Y, Xiao L. (2011): Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clin. Microbiol. Rev*., 24(1): 110 – 140. DOI: 10.1128/CMR.00033-10

Fogt-Wyrwas, R., Jarosz, W., Mizgajska-Wiktor, H. (2007): Utilizing a polymerase chain reaction method for the detection of *Toxocara canis* and *T. cati* eggs in soil. *J. Helminthol*., 81: 75 – 78. DOI: 10.1017/S0022149X07241872

Greene, C.E. (2011): *Infectious diseases of the dog and cat*. Print Saunders, 1376 pp.

Grecca M., Thomaz-Soccol V., Costa M., Silvia R., Osaki K. (2013): Occurrence of *Cryptosporidium* sp. in dogs and cats from curitiba and its metropolitan area. *Arch. Vet. Sci*., 18(3): 01 – 06. DOI:10.5380/avs.v18i3.29522

Hamnes, I., Gjerde, B., Robertson, L. (2007): A longitudinal study on the occurrence of *Cryptosporidium* and *Giardia* in dogs during their first year of life. *Acta Vet. Scand*., 49(1): 22. DOI: 10.1186/1751-0147-49-22

Joffe, D., Van Niekerk, D., Gagné, F., Gilleard, J., Kutz, S., Lobinger, R. (2011): The prevalence of intestinal parasites in dogs and cats in Calgary, Alberta. *Can. Vet. J*., 52(12): 1323 – 1328

Konyaev, S.V., Borcova, M.S. (2015): Giardiasis (Giardia) of dogs in Russia: the prevalence and effective treatment. *RVJ*, 5: 42 – 44 (In Russian)

Kotelnikov, G.A (1974): Diagnostics of animal helminthiasis. Moscow, Koloss, 240 pp. (In Russian)

Kotelnikov, G.A, Chrenov, V.M. (1980): Guidelines for Diagnostic Frequency Helminthiasis in Farm Animals. Moscow (In Russian)

Kurnosova, O.P. (2014): Distribution of protozoa *Lamblia* (*Giardia*) sp. among dogs and cats of Moscow. *Med. Parasitol*.,(3): 23 – 25 (In Russian)

Kurnosova, O.P., Odoyevskaya, I.M. (2017): Toxocariasis in pet dogs and cats. *Med. Parasitol*., 4: 30 – 34 (In Russian)

Kurnosova, O.P. (2009): Parasitic diseases in domestic dogs and cats in metropolitan Moscow. *Med. Parasitol*., 4: 31 – 35 (In Russian)

Laboratory diagnosis of helminthiases and protozoosis: MUC 4.2.3145-13, Moscow (In Russian)

Levecke, B., Dreesen, L., Dorny, P., Verweij, J., Vercammen, F., Casaert, S., Vercruysse, J., Geldhof, P. (2010): Molecular Identification of *Entamoeba* spp. In Captive Nonhuman Primates. *Clin. Vet. Microbiol*., (48): 2988–2990. DOI: 10.1128/JCM.00013-10

Loshchinin, M.N., Studennikova, U.V., Belimenko, V.V. (2015): Intestinal protozooses of dogs and cats in urbanized ecosystems. *Russian Vet. J*., 1: 20 – 21 (in Russian)

Malysheva, N.S., Samofalova, N.A., Grigoriev, D.G., Vagin, N.A., Elizarov, A.S., Gladkikh, K.A., Shuikina, E.E. (2013): The problem of toxocariasis in the current situation and improvements in approaches to its prevention. Scientific Notes: Electronic scientific journal of the Kursk State University, 1: 25 – 30 (In Russian)

Mikhina, N.V. (2008): Epizootology, pathomorphology and improvements in the therapy of helminthiases of cats. Author's abstract of a dissertation for the award of the degree of Candidate of Veterinary Sciences, Moscow (In Russian)

Panova, O.A. (2011): Toxocariasis of carnivorous: diagnostic methods and bioecological aspects of development of pathogens in a metropolis. Authoref. Dis. Cand. Biol. Sci. Moscow (In Russian)

Paradies, P., Iarussi, F., Sasanelli, M., Capogna, A., Lia, R., Zucca, D., Greco, B., Cantacessi, C., Otranto, D. (2017): Occurrence of strongyloidiasis in privately owned and sheltered dogs: clinical presentation and treatment outcome. *Parasit. Vectors*, (10): 345. DOI: 10.1186/s13071-017-2275-5

Pereira, A. , Martins, A. , Brancal, H. , Vilhena, H., Silva, P., Pimenta, P., Diz-Lopes, D., Neves, N., Coimbra, M., Alves, F., Cardoso, L., Maia, K.(2016): Parasitic zoonoses associated with dogs and cats: a survey of Portuguese pet owners' awareness and deworming practices. *Parasit. Vectors*, 9: 245. DOI: 10.1186/s13071-016-1533-2

Prozorov, A.M. (1999): Parasitic diseases of dogs and cats in St. Petersburg. Author's abstract of a dissertation for the award of the degree of Candidate of Biological Sciences, St. Petersburg (In Russian)

Regan, C.S., Yon, L., Hossain, M., Elsheikha, M. (2014): Prevalence of *Entamoeba* species in captive primates in zoological gardens in the UK. PeerJ., (2): 492. DOI: 10.7717/peerj.492

Sergiev, V.P., Lobzin, Yu.V., Kozlov, S.S. (2006): Human parasitic diseases. S. Petersburg, 240 pp. (In Russian)

Shcheveleva, T.N., Sofyin, V.S., Mironova, N.I., Karakotin, A.A. (2016): The epidemiologic characteristics of toxocariasis (review of literature and own research). Scientific review. *Med. Sci.*, 6: 124 – 128 (In Russian)

Stepanov, V.A. (2014): Improvements in measures against parasitic diseases of carnivorous animals (pharmacological and toxicological properties of new topical drugs). Author's abstract of a dissertation for the award of the degree of Candidate of Veterinary Sciences, Moscow (In Russian)

Thompson, R.C., Monis, P.T. (2004): Variation in *Giardia*: implications for taxonomy and epidemiology. *Adv. Parasitol*., (58): 137 – 141. DOI: 10.1016/S0065-308X(04)58002-8

Thompson, R.C., Palmer, C.S., O'Handley, R. (2008): The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. *Vet. J*., 177(1): 18 – 25. DOI: 10.1016/j.tvjl.2007.09.022

Umur, S., Meral, Y , Bolukbas, C., Gurler, A., Acici, M. (2017): First clinical *Strongyloides stercoralis* case in a dog in Turkey. *Turk. J. Vet. Anim. Sci*., 4(1): 312 – 315. DOI: 10.3906/vet-1606-2

Upjohn, M., Cobb, C., Monger, J.,Geurden, T., Claerebout, E., Fox, M. (2010): Prevalence, molecular typing and risk factor analysis for *Giardia duodenalis* infections in dogs in a central London rescue shelter. *Vet. Parasitol*., 172(3 – 4): 341 – 346. DOI: 10.1016/j.vetpar.2010.05.010

Uspensky, A.V., Peshkov, R.A Gorokhov, V.V., Gorokhova, E.V. (2011): Toxocariasis in the current context. *Med. Parasitol*., 2: 3 – 6 (In Russian)

Verweij J., Brienen E. (2003): *Entamoeba histolytica* infections in captive primates. *Parasitol. Res*., 90(2): 100 – 103 DOI: 10.1007/s00436-002-0808-z

Zubareva, I.M. (2001): The main helminthiases of domestic carnivores in large cities (e.g. Novosibirsk). Author's abstract of a dissertation for the award of the degree of Candidate of Veterinary Sciences, Novosibirsk (In Russian)

DOI 10.2478/helm-2019-0008

HELMINTHOLOGIA, 56, 2: 118 – 123, 2019

Detection and identification of *Toxocara canis* in infected dogs using PCR

H. ÖGE*, S. ÖGE, G. ÖZBAKIŞ-BECERİKLİSOY

Ankara University, Faculty of Veterinary Medicine, Department of Parasitology, Ankara, Turkey, *E-mail: *hoge@ankara.edu.tr*

Article info

Received September 26, 2018
Accepted December 18, 2018

Summary

Faecal samples were collected from 224 dogs (47 villages) in Ankara. *Toxocara* spp. eggs were diagnosed in faeces using centrifugal flotation and sedimentation methods. A total of 21 dogs (9.38 %) were positive for *Toxocara* spp. eggs. In this study, we used the PCR technique that, in combination with DNA sequencing, allows the detection and identification of *T.canis* eggs in faeces of infected dogs. For this purpose, the ATPase subunit-6 gene (mtDNA) was selected as a target for the amplification *T. canis*. The primers were used to amplify 217 bp region. Amongst 21 coproscopically detected *Toxocara* isolates from dogs, 5 (23.8 %) samples were PCR-positive for *T. canis*, and the remaining 16 samples were PCR-negative. Results indicate that PCR can detect *Toxocara canis* DNA in faeces of infected dogs, but efficacy was low when compare to sedimentation/flotation. PCR is additional test for diagnosing of this infection. But, the difficulties of identification based on PCR in faecal examinations need to be investigated further.
Keywords: *Toxocara canis*; dog; faecal examination; ATPase subunit-6

Introduction

Toxocariasis is a zoonosis with worldwide distribution caused by *Toxocara* species of dogs and cats. VLM in humans occurs primarily because of the ingestion of infective eggs (Macpherson, 2013; Strube *et al.,* 2013). The ways of transmission to humans are as follows; soil and sandpits contamination in children, geophagia (Overgaauw & Nederland, 1997; Macpherson, 2005; Bowman, 2009), ingestion of eggs contain infective larvae from dog's coat (Amaral *et al.,* 2010; Macpherson, 2013; Öge *et al.,* 2014), consumption of unwashed raw vegetables or fruits (Kozan *et al.,* 2005; Lee *et al.,* 2010), consumption of raw or undercooked meat containing arrested infective larvae in paratenic host (Lee *et al.,* 2010; Macpherson, 2013; Strube *et al.,* 2013), low socio-economic level and failure to regularly pick up and dispose of faeces (Overgaauw & Nederland, 1997; Robertson & Thompson, 2002).

In different countries, the prevalence of *T. canis* ranged between 4.4 % and 33.8 % in dogs (Habluetzel *et al.,* 2003; Sager *et al.,* 2006; Sowemimo, 2007; Claerebout *et al.,* 2009; Soriano *et al.,* 2010). The prevalence of *T. canis* varied from 4.2 % to 47.8 % in Turkey (Yıldırım *et al.,* 2007; Kozan *et al.,* 2007; Ünlü & Eren, 2010; Çiçek & Yılmaz, 2012). In dogs, routine diagnosis relies mainly on detection of eggs of the parasite in faeces. However, *T. canis* and *T. cati* are not to be clearly distinguishable by microscopy and serological diagnostic methods. The accurate identification of these species and differentiation from each other have an important role for investigating their life-cycles, epidemiology and specific diagnosis of toxocariasis.

The seroprevalence of human toxocariasis varies from 2.4 % to 92.8 % (Rubinsky-Elefant *et al.,* 2010). In Turkey, the prevalence was found to be 7.6 – 26.42 % in recent years (Kustimur *et al.,* 2007; Karadam *et al.,* 2008; Akdemir, 2010; Çiçek & Yılmaz, 2012).

* – corresponding author

PCR is used for rapid and specific diagnosis, because of their ability to specifically amplify DNA from nematode eggs and thinly sections of larvae or adult stages (Gasser, 2013; Smith *et al.,* 2009). Primer design is the key step in PCR for the identification of parasites. ITS-1 and ITS-2 of nuclear rDNA sequences have been demonstrated to provide reliable genetic markers for the identification and differentiation of species of *Toxocara* and related nematodes (Jacobs *et al,.* 1997; Zhu *et al.,* 1998; Li *et al.,* 2007). In addition to nuclear ITS-1 and ITS-2 rDNA sequences, recent studies have shown that mtDNA useful alternative genetic markers for investigating of parasitic nematodes (Gasser, 2013). Various mitochondrial DNA regions have been employed for studying the population genetics of parasitic nematodes (Li *et al.,* 2008; Wickramasinghe *et al.,* 2009; Gasser, 2013). However, there is still limited information on the mt genomes of socioeconomically important *Toxocara* parasites. Therefore, there is need a suitable DNA target region (genetic marker) for the accurate identification of *T. canis* by PCR technique.

In addition to conventional methods, this coprological study was undertaken to detection and identification of *Toxocara canis* in infected dogs using PCR in the region of Ankara.

Materials and Methods

Sample collection and faecal analysis

Faecal samples taken from 224 dogs in 47 villages were investigated for *Toxocara* eggs. Age and sex of the dog were determined. Faecal samples were collected from dogs either within the area accessed by free or tethered dogs. For safety reasons, samples were frozen at -80°C for 10 days before examination. Faecal samples were examined by sedimentation-formalin-ethyl acetate and centrifugal flotation with $ZnSO_4$-solution (Truant *et al.,* 1981). Eggs from all the *Toxocara* positive samples detected by flotation/sedimentation were examined by one step PCR to determine the *T. canis*. To concentrate eggs, faecal samples were prepared with improved flotation method (Szell *et al.,* 2014). When *Toxocara*-type eggs were detected microscopically, 1 ml of the upper part of the flotation from the centrifuge tube was transferred to the 15 ml falcon tube. This step was repeated 4 times. The tube was filled with water and centrifuged at 2000 x g 10 min. The supernatant was discarded, the sediment resuspended in 1 ml water, transferred to a 2ml micro tube and these sediment was used for DNA purification.

DNA isolation and PCR

For the PCR, *Toxocara* egg DNA was extracted from faeces by QIAmp DNA Stool Mini kit (Qiagen), according to the manufacturer's instructions with the following modifications: The samples (sediment above defined) were subjected to 95°C, 30 min in Buffer ASL, and proteinase K digestion was performed 70°C, 30 min. Final dilution of DNA were made in 80 µl of elution buffer and stored at -20°C until using. The concentration of DNA in each sample was measured by a spectrophotometer (Thermoscientific Nanodrop-ND 2000) for qualitative and quantitative analyses. The isolates of eggs and adult of *T. canis* prepared in Özbakış' work (2015) were used for positive control sample. Also, distilled water was used as a negative-control.

A forward (*T.canis* ATP-F1: GTTTGTTGTTTTGGGGGCTA) and reverse (*T.canis* ATP-R1: CCAAAGGACGAGAAACCTCA) primers were used to amplify a 217 bp region of the ATP synthase subunit 6 gene of *T. canis* (Özbakış, 2015). PCR was carried out in a 30 µl total volume mix containing 10xTaq buffer (1.25 Ml including $(NH_4)_2SO_4$), 25 mmol/L of $MgCl_2$, 10mM of dNTP mix, 5U/µl Taq DNA polymerase (Fermentas, Waltham, MA, USA), water (18Mohm-cm, AppliChem, Darmstadt, Germany), 10 pmol/µl primers and 10 µl of template DNA. The reaction conditions were: 5 min at 94 °C, followed by 34 cycles of 30 s denaturation at 94 °C, 1 min annealing at 50 °C, 1 min extension at 72 °C and 10 min final extension at 72°C, using a thermal cycler (PX2 Thermo, USA). Amplicons were detected on a 3% agarose TAE gel with ethidium bromide-stained. It was visualized under the UV light with gel imaging system (NDR Bio-Imaging systems Mini Bis Pro).

Sequencing analysis

Toxocara egg products in 3 of PCR positive dogs were subjected to DNA sequencing. Subsequently, amplicons were clean-upped by High Pure PCR Clean-up microcit (Roche, Germany). Nucleotide sequence analysis was performed by Sentegen Biotechnolo-

Comparison of *Toxocara* egg prevalence in dogs' faeces/hair, soil, raw vegetables, and anti-*Toxocara* IgG positivity in human in Ankara, TURKEY

Province	Dog		Soil (%)	Vegetables (%)	Human (%)	References
	Faeces (%)	Hair (%)				
Ankara/TURKEY	**9.38**					**Present study**
	5	14				Öge *et al.,* 2014
		49				Öge *et al.,* 2013
			30.6			Oge & Oge, 2000
			15.05			Avcioglu & Burgu, 2008
				1.5		Kozan *et al.,* 2005
					9.7	Kustimur *et al.,* 2007

gy in Ankara and undertaken by BLAST algorithms and databases from the National Centre for Biotechnology (http://www.ncbi.nlm.nih.gov).

Phylogenetic analysis was performed in the Mega software (version 6.0) (Tamura *et al.,* 2013). The tree was constructed using neighbour-joining method (Saitou & Nei, 1987) based on Kimura 2-parameter model (Kimura, 1980) in the software package program. Bootstrap resampling was calculated from 1000 pseudo replicates with random seeds (Felsenstein, 1985).

Ethical Approval and/or Informed Consent

For this study formal consent is not required.

Results and Discussion

Toxocara eggs were detected in 21 of 224 dogs (9.38 %). *Toxocara canis* was identified in 5 (23.8 %) of the 21 *Toxocara* egg-positive samples by PCR (Fig. 1). But, efficacy of PCR was low when compare to sedimentation/flotation. The conventional parasitological examination is routinely used for diagnosis of toxocariasis in field. PCR has used for identification and differentiation of *Toxocara* species. The difference between *T. canis* and *T. cati* in ITS-1 and/or ITS-2 of nuclear ribosomal DNA (rDNA) have been demonstrated by various authors (Jacobs *et al.,* 1997; Zhu *et al.,* 1998; Li *et al.,* 2007; Borecka *et al.,* 2008; Wickramasinghe *et al.,* 2009; Fahrion *et al.,* 2011). Recent studies have shown that sequences derived from the mtDNA genes provide alternative genetic marker for investigating genetic structures, systematics and phylogeny of parasitic nematodes (Wickramasinghe *et al.,* 2009; Gasser, 2013). Mitochondrial DNA (mtDNA) markers can be used for investigating the taxonomy and genetics of *Toxocara* species. Conserved primers can be rationally and selectively designed in mitochondrial genome. Wickramasinghe *et al.* (2009) reported that the mitochondrial ATPase 6 genes were well conserved in *Toxocara* species and can be used for discrimination of species and for molecular phylogenetic. The important finding in this study was that only 5 out of 21 microscopically positive samples were PCR-positive *T.canis*. The other 16 dogs that were PCR negative were microscopically positive for *Toxocara* eggs. PCR efficacy depends on the number of eggs in faeces. PCR may not be able to detect DNA of ascarids as a result of low DNA concentration. We detected that the number of eggs in these faecal samples (epg) was very low in dogs (< 50). *Toxocara* species have the host specificity, i.e. *T. cati* for felids and *T. canis* for canids. But, Roth & Schneider (1971) reported the findings of *T. canis* adults in the intestines of dissected cats. Some studies have suggested that coprophagy in dogs may be responsible for finding eggs of dog-typical (Sager *et al.,* 2006) as well as dog-atypical (Fahrion *et al.,* 2011). Dogs may consume their own faeces, faeces of other dogs and/or faeces of other species (Nijsse *et al.,* 2014). Looking at the PCR results, in some dogs might have *T. cati* parasites instead of *T. canis*. But, this situation generally can not be explanation for the low PCR efficacy. That's why the result of faecal examination must be interpreted with carefully. The development of molecular diagnostic tests for identifying *T. canis* is important. Mainly *T. cati* resemble *T. canis* very closely in routine microscopic diagnosis and this may be lead to miss-identification. *Toxocara cati* might play a role in human toxocariasis than estimated rate, as there is no difference in the zoonotic potential of *T. canis* and *T. cati* (Oge *et al.,* 2014).

The phylogenetic tree based on ATPase subunit-6 gene sequences was able to distinguish between ascarid nematode samples and was used a *Haemonchus contortus* for an out group (Fig. 2). 244-407 bp were used in reference to FJ418787 accession number of *T. canis* gene data compared to phylogenetic tree. When the sequences data were compared with obtained *T. canis* sequences from GenBank database (Access. no: KJ777173, KJ777174, FJ418787, EU730761, JN593098) on ATPase subunit 6 gene between 98.2 % and 99.4 % homology exhibited. The identity between our *T.canis* samples and the reference *T.canis* samples was

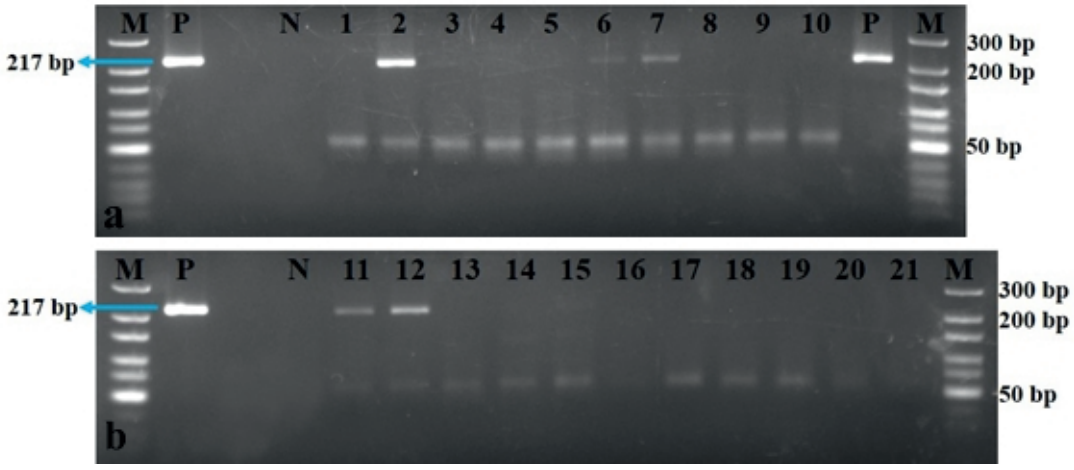

Fig. 1. Analysis of PCR products amplified of *T. canis* from faecal samples by agarose gel electrophoresis.
M: Marker, PK: Positive control, NK: no DNA control, 1 - 21: Dog isolates

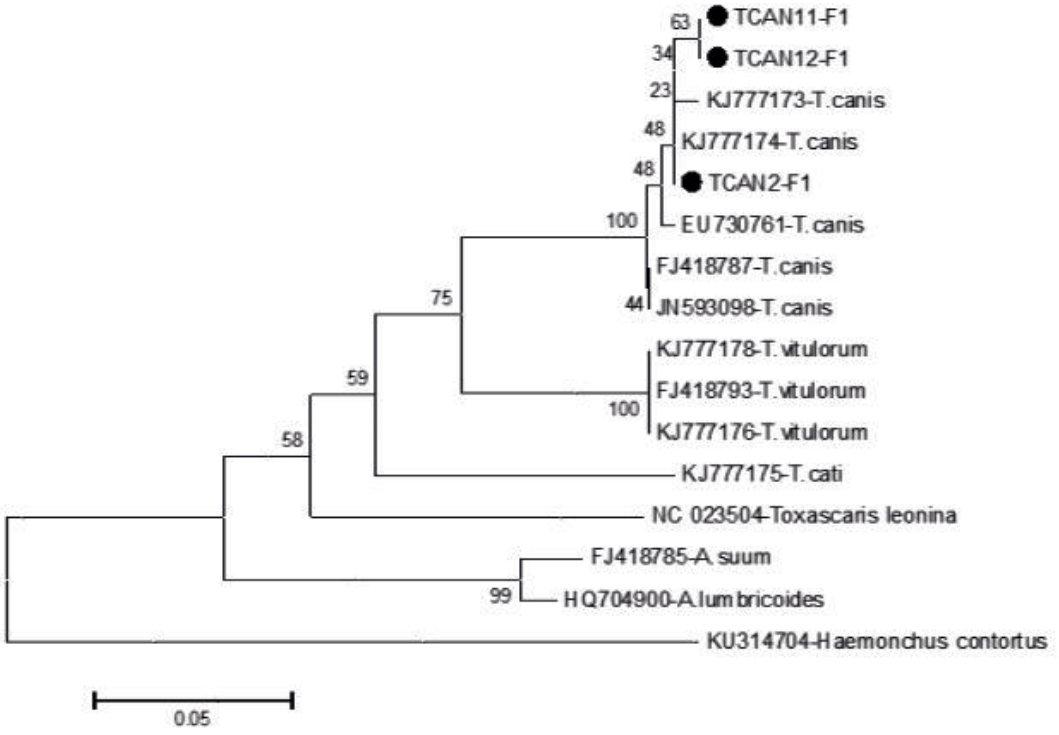

Fig. 2. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length= 0.66427549 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances are in the units of the number of base substitutions per site.

low (98.2 %) considering it was such a small fragment (164 bp). Nucleotide sequence identities with each other of our samples (11, 12 and 2 number dog isolates) were found 100 %, 100 % and 94.4 %, respectively.

Based on faecal analysis, *Toxocara* spp. eggs were found in the faeces of 9.38 % of investigated dogs. The low prevalence of *Toxocara* infection in dogs in these villages could be attributed to the fact that the majority of dogs which were older than 2 years when looked at the raw data. *Toxocara* egg has been found in both young (5/43-11.62 %) and adult (16/181-8.83 %) animals. Adult dogs may still pose a risk to human health as they are susceptible to *Toxocara* infection.

The potential role of *Toxocara* parasites in human toxocariasis should not be ignored or underestimated. The presence of *Toxocara* spp. eggs was found in the soil, raw vegetables and dogs' faeces and hair in Ankara (Table 1). These situations are significant as the eggs have the potential to develop to infective larval stage and responsible for most VLM cases in humans. In the current study, the prevalence of *Toxocara* eggs in faeces is not high when compared with similar studies is not high when compared with similar studies (Öncel, 2004; Orhun & Ayaz, 2006; Kozan *et al.,* 2007; Ünlü & Eren, 2007; Balkaya & Avcıoğlu, 2011) but may be important.

The findings and considerations presented here indicate that we may have an imprecise image of true prevalence of patent infections with *Toxocara* spp. in dogs. The difficulties of identification based on PCR in faecal examinations need to be investigated further.

Conflict of Interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Akdemir, C. (2010): Visceral larva migrans among children in Kütahya (Turkey) and an evaluation of playgrounds for *T. canis* eggs. *Turk. J. Pediatr.,* 52(2): 158 – 162.

Amaral, H.L.C., Rassier, G.L., Pepe, M.S., Gallina, T., Villela, M.M., Nobre, M.O., Scaini, C.J., Berne, M.E.A. (2010): Presence of *Toxocara canis* eggs on the hair of dogs: A risk factor for Visceral Larva Migrans. *Vet. Parasitol.*, 174(1-2): 115 – 118. DOI: 10.1016/j.vetpar.2010.07. 016

Avcioglu, H., Burgu, A. (2008): Seasonal prevalence of *Toxocara* ova in soil samples from public parks in Ankara, Turkey. *Vector Borne Zoonotic Dis.,* 8(3): 345 – 350. DOI: 10.1089/vbz. 2007.0212

Balkaya, İ., Avcioğlu, H. (2011): Gastro-intestinal helminths detected by coprological examination in stray dogs in the Erzurum province-Turkey. *Kafkas Univ. Vet. Fak. Derg.,* 17(Suppl A): S43 – S46.

Borecka, A., Gawor, J., Niedworok, M., Sordyl, B. (2008): Detection of *Toxocara canis* larvae by PCR in the liver of experimentally infected Mongolian gerbils (*Meriones unguiculatus*). *Helmintholo-gia* 45(3): 147 – 149. DOI: 10.2478/s11687-008-0029-9

Bowman, D.D. (2009): Georgis' Parasitology for Veterinarians. 9th Edition, St Louis-Missouri, USA, Saunders Elsevier

Claerebout, E., Casaert, S., Dalemans, A.C., De Wilde, N., Levecke, B., Vercruysse, J., Geurden, T. (2009): Giardia and other intestinal parasites in different dog populations in Northern Belgium. *Vet. Parasitol.*, 161(1-2): 41 – 46. DOI: 10.1016/j.vetpar.2008.11.024

Çiçek, M., Yilmaz, H. (2012): Prevalence of toxocariasis in human and dogs in Van province. *Kafkas Univ. Vet. Fak. Derg.,* 18(4): 531 – 536. DOI: 10.9775/kvfd.2011.5211

Fahrion, A.S., Schnyder, M., Wichert, B., Deplazes, P. (2011): *Toxocara* eggs shed by dogs and cats and their molecular and morphometric species-specific identification: Is the finding of *T. cati* eggs shed by dogs of epidemiological relevance? *Vet. Parasitol.,* 177(1-2): 186 – 189. DOI: 10.1016/j.vetpar.2010.11.028

Felsenstein, J. (1985): Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39: 783 – 791. DOI: 10.1111/j.1558-5646.1985.tb00420.x

Gasser, R.B. (2013): A perfect time to harness advanced molecular technologies to explore the fundamental biology of *Toxocara* species. *Vet. Parasitol.*, 193(4): 353 – 364. DOI: 10.1016/j.vetpar.2012.12.031

Habluetzel, A., Traldi, G., Ruggieri, S., Attili, A.R., Scuppa, P., Marchetti, R., Menghini, G., Esposito, F. (2003): An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy. *Vet. Parasitol.*, 113(3 – 4): 243 – 252. DOI: 10.1016/S0304-4017(03)00082-7

Jacobs, D.E., Zhu, X., Gasser, R.B., Chilton, N.B. (1997): PCR-based methods for identification of potentially zoonotic ascaridoid parasites of the dog, fox and cat. *Acta Trop.*, 68(2): 191 – 200. DOI: 10.1016/S0001-706X(97)00093-4

Karadam, S.Y., Ertug, S., Ertabaklar, H., Okyay, P. (2008): The comparison of IgG antibodies specific to *Toxocara* spp. among eosinophilic and non-eosinophilic groups. *New Microbiol.*, 31: 113 – 116

Kimura, M. (1980): A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evolution*, 16: 111 – 120

Kozan, E., Gonenc, B., Sarimehmetoglu, O., Aycicek, H. (2005): Prevalence of helminth eggs on raw vegetables used for salads. *Food Control* 16(3): 239 – 242. DOI: 10.1016/j.foodcont.2004.02.005

Kozan, E., Kircali Sevimli, F., Birdane, F.M. (2007): The occurrence of gastrointestinal cestode and nematode infections in stray dogs in Afyonkarahisar and Eskisehir provinces. *Türkiye Parazitol Derg.*, 31(3): 208 – 211

Kustimur, S., Doğruman Al, F., Oguzulgen, K., Bakir, H., Maral, I., Türktas, H., Tüzün, H. (2007): *Toxocara s*eroprevalence in adults with bronchial asthma. *Trans. R. Soc. Trop. Med. Hyg.*, 101: 270 – 274. DOI: 10.1016/j.trstmh.2006.08.013

Lee, A.C.Y., Schantz, P.M., Kazacos, K.R., Montgomery, S.P., Bowman, D.D. (2010): Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. *Trends Parasitol.*, 26(4): 155 – 161. DOI: 10.1016/j.pt.2010.01.002

Li, M.W., Lin, R.Q., Chen, H.H., Sani, R.A., Song, H.Q., Zhu, X.Q. (2007): PCR tools for the verification of the specific identity of ascaridoid nematodes from dogs and cats. *Mol. Cell. Probes.*, 21(5-6): 349 – 354. DOI: 10.1016/j.mcp.2007.04.004

Li, M.W., Lin, R.Q., Song, H.Q., Wu, X.Y., Zhu, X.Q. (2008): The complete mitochondrial genomes for three *Toxocara* species of human and animal health significance. *BMC Genomics*. 9: 224 – 233. DOI: 10.1186/1471-2164-9-224

Macpherson, C.N.L. (2005): Human behaviour and the epidemiology of parasitic zoonoses. *Int. J. Parasitol.*, 35(11-12): 1319 – 1331. DOI: 10.1016/j.ijpara.2005.06. 004

Macpherson, C.N.L. (2013): The epidemiology and public health importance of toxocariasis: A zoonosis of global importance. *Int. J. Parasitol.*, 43(12-13): 999 – 1008. DOI: 10.1016/j.ijpara.2013.07.004

Nijsse, R., Mughini-Gras, L., Wagenaar, J.A., Ploeger, H.W. (2014): Coprophagy in dogs interferes in the diagnosis of parasitic infections by faecal examination. *Vet. Parasitol.*, 204(3-4): 304 – 309. DOI: 10.1016/j.vetpar.2014.05.019.

Oge, S., Oge, H. (2000): Prevalence of *Toxocara* spp. eggs in the soil of public parks in Ankara, Turkey. *Dtsch. Tierarztl. Wochenschr.*, 107: 72 – 75.

Orhun, R., Ayaz, E. (2006): Prevalence of helminths in dogs in the region of Van and their potential public health significance. *Türkiye Parazitol. Derg.,* 30(2): 103 – 107.

Overgaauw, P.A.M., Nederland, V. (1997): Aspects of *Toxocara* epidemiology: toxocarosis in the human. *Crit. Rev. Microbiol.*, 23(3): 215 – 231. DOI: 10.3109/10408419709115137

Öge, S., Öge, H., Gönenç, B., Özbakiş, G., Aşti, C. (2013): Presence of *Toxocara* eggs on the hair of dogs and cats. *Ankara Üniv. Vet. Fak. Derg.*, 60: 171 – 176

Öge, H., Öge, S., Özbakiş, G., Gürcan, S. (2014): Comparison of *Toxocara* eggs in hair and faecal samples from owned dogs and cats collected in Ankara, Turkey. *Vet. Parasitol.*, 206 (3-4): 227 – 231. DOI: 10.1016/j.vetpar.2014.10.005

Öncel, T. (2004): Toxocariosis canis in domestic dogs in İstanbul, Turkey. *Kafkas Üniv. Vet. Fak. Derg.,* 10(2): 151 – 153.

Özbakiş, G. (2015): PCR detection of Visceral Larvae Migrans in mice experimentally infected with *Toxocara canis*. PhD Thesis, Ankara, Turkey: The Graduate School of Health Sciences of Ankara University

Robertson, I.D., Thompson, R.C. (2002): Enteric parasitic zoonoses of domesticated dogs and cats. *Microbes Infect.*, 4(8): 867 – 873. DOI: 10.1016/S1286-4579(02)01607-6

Roth, B., Schneider, C.C. (1971): Untersuchungen zur Abhangigkeiten des "weissen Blutbildes" bei Hauskatzen (*Felis domestica*) von Wurminfektionen des Darmes. [Relation of the white blood picture to intestinal helminth infestations in cats (*Felis domestica*)]. *Berl. Munch. Tierarztl. Wochenschr.,* 84(22): 436 – 437 (In German)

Rubinsky-Elefant, G., Hirata, C.E., Yamamoto, H.J., Ferreira, M.U. (2010): Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular form. *Ann. Trop. Med. Parasitol.*, 104(1): 3 – 23. DOI: 10.1179/136485910X12607012373957.

Sager, H., Moret, C.H.S., Grimm, F., Deplazes, P., Doherr, M.G., Gottstein, B. (2006): Coprological study on intestinal helminths in Swiss dogs: temporal aspects of anthelminthic treatment. *Parasitol. Res.,* 98(4): 333 – 338. DOI: https://doi.org/10.1007/s00436-005-0093-8

Saitou, N., Nei, M. (1987): The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406 – 425. DOI: 10.1093/oxfordjournals.molbev.a040454

Smith, H., Holland, C., Taylor, M., Magnaval, J.F., Schantz, P., Maizels, R. (2009): How common is human toxocariasis? Towards standardizing our knowledge. *Trends Parasitol.*, 25(4): 182 – 188. DOI: 10.1016/j.pt.2009.01.006

Soriano, S.V., Pierangeli, N.B., Roccia, I., Bergagna, H.F.J., Lazzarini, L.E., Celescinco, A., Saiz, M.S., Kossman, A., Contreras, P.A., Arias, C., Basualdo, J.A. (2010): A wide diversity of zoonotic intestinal parasites infects urban and rural dogs in Neuquen, Patagonia, Argentina. *Vet. Parasitol.*, 167(1): 81 – 85. DOI: 10.1016/j.vetpar.2009.09.048

Sowemimo, O.A. (2007): Prevalence and intensity of *Toxocara canis* (Werner, 1782) in dogs and its potential public health significance in Ile-Ife, *Nigeria. J. Helminthol.*, 81(4): 433 – 438. DOI: 10.1017/S0022149X07850267

Strube, C., Heuer, L., Janecek, E. (2013): *Toxocara* spp. infections in paratenic hosts. *Vet. Parasitol.*, 193(4): 375 – 389. DOI: 10.1016/j.vetpar.2012.12.033

Szell, Z., Sreter-Lancz, Z., Sreter, T. (2014): Evaluation of faecal flotation methods followed by species-specific PCR for detection of *Echinococcus multilocularis* in the definitive hosts. *Acta Parasitol.*, 59(2): 331 – 336. DOI: 10.2478/s11686-014-0248-9

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.*, 30: 2725 – 2729. DOI: 10.1093/molbev/mst197

Truant, A.A., Elliot, S.H., Kelly, M.T., Smith, J.H. (1981): Comparison of formalin-ethyl ether sedimentation, formalin-ethyl acetate sedimentation, and zinc sulphate flotation techniques for detection of intestinal parasites. *J. Clin. Microbiol.*, 13(5): 882 – 884

Ünlü, H., Eren, H. (2007): Gastro-intestinal helminths detected by fecal examination in stray dogs in the Aydin province. *Türkiye Parazitol. Derg.,* 31(1): 46 – 50

Yildirim, A., İça, A., Düzlü, O., Yavuz, A., İnci, A. (2007): The prevalence of gastrointestinal helminths in dogs by coprological examination in Kayseri province. *Erciyes Üniv. Vet. Fak. Derg.,* 4(2): 65 – 71

Wickramasinghe, S., Yatawara, L., Rajapakse, R.P.V.J., Agatsuma, T. (2009): *Toxocara canis* and *Toxocara vitulorum*: molecular characterization, discrimination, and phylogenetic analysis based on mitochondrial (ATP synthase subunit 6 and 12S) and nuclear ribosomal (ITS-2 and 28S) genes. *Parasitol. Res.*, 104: 1425 – 1430. DOI: 10.1007/s00436-009-1345-9

Zhu, X.Q., Jacobs, D.E., Chilton, N.B., Sani, R.A., Cheng, N.A.B.Y., Gasser, R.B. (1998): Molecular characterization of a *Toxocara* variant from cats in Kuala Lumpur, Malaysia. *Parasitology* 117: 155 – 164

DOI 10.2478/helm-2019-0002

HELMINTHOLOGIA, 56, 2: 124 – 131, 2019

Heliconema monopteri n. sp. (Nematoda: Physalopteridae) from *Monopterus cuchia* (Hamilton) (Osteichthyes: Synbranchidae) in India, with notes on the taxonomy of *Heliconema* spp.

F. MORAVEC[1]*, A. CHAUDHARY[2], H. S. SINGH[2]

[1]Institute of Parasitology, Biology Centre, Czech Academy of Sciences (CAS), Branišovská 31, 370 05 České Budějovice, Czech Republic, *E-mail: *moravec@paru.cas.cz*; [2]Department of Zoology, Chaudhary Charan Singh University, Meerut (UP) - 250004, India

Article info

Received December 11, 2018
Accepted January 17, 2019

Summary

A new nematode species, *Heliconema monopteri* n. sp. (Physalopteridae), is described from the stomach and intestine of the freshwater fish *Monopterus cuchia* (Hamilton) (Synbranchidae) in Bijnor district, Uttar Pradesh, India. It is mainly characterized by the lengths of spicules (468 – 510 µm and 186 – 225 µm), the postequatorial vulva without elevated lips, the presence of pseudolabial lateroterminal depressions and by the number and arrangement of caudal papillae. This is the first representative of the genus reported from a synbranchiform fish. Another new congeneric species, *Heliconema pisodonophidis* n. sp. is established based on a re-examination of nematodes previously reported as *H. longissimum* (Ortlepp, 1922) from *Pisodonophis boro* (Hamilton) (Ophichthidae) in Thailand; ovoviviparity in this species is a unique feature among all physalopterids. *Heliconema hamiltonii* Bilqees et Khanum, 1970 is designated as a *species dubia* and the nematodes previously reported as *H. longissimum* from *Mastacembelus armatus* (Lacépède) in India are considered to belong to *H. kherai* Gupta et Duggal, 1989. A key to species of *Heliconema* Travassos, 1919 is provided.

Keywords: parasitic nematode; Physalopteroidea; new species; freshwater fish; Synbranchiformes; South Asia

Introduction

Recent parasitological examinations of some freshwater fishes in Bijnor district, Uttar Pradesh, India carried out in August 2018, revealed the presence of physalopterid nematodes in the digestive tract of the cuchia *Monopterus cuchia* (Hamilton) (Synbranchidae, Synbranchiformes). A detailed study of their morphology by both light (LM) and scanning electron microscopy (SEM) shows that they represent a new species of the genus *Heliconema* Travassos, 1919, which is described below.

The cuchia (maximum length 70 cm) is a tropical commercial fish that occurs in freshwater and brackish-water habitats of South Asia (Pakistan, India, Nepal, Bangladesh and Myanmar). Adults are known to hibernate in mud during cold season (Froese and Pauly, 2018).

Material and Methods

Fish were obtained from the fish market in Bijnor (fish allegedly caught in the River Ganga), Bijnor district (29.3724°N, 78.1358°E), Uttar Pradesh, India in August 2018 and these were kept alive in aquaria at 24 °C until dissection. A total of two specimens of *Monopterus cuchia* (total body length 37 and 45 cm) were examined for the presence of parasites. The nematodes obtained were washed in physiological saline and then fixed in hot 70% ethanol. For LM, the nematodes were cleared using glycerine. Drawings

* – corresponding author

were made with the aid of a Zeiss drawing attachment. Specimens used for SEM were postfixed in 1 % osmium tetroxide (in phosphate buffer), dehydrated through a graded acetone series, critical-point-dried and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope at an accelerating voltage of 4 kV (GB low mode). All measurements are in micrometres unless otherwise indicated. For comparison, specimens of *Heliconema* from *Pisodonophis boro* (Hamilton) in Thailand, identified by Moravec *et al*. (2007) as *H. longissimum* (Ortlepp, 1923) and deposited in the Helminthological Collection of the Institute of Parasitology, CAS, České Budějovice (Cat. No. N – 862), were re-examined. The fish nomenclature adopted follows FishBase (Froese and Pauly, 2018).

Ethical Approval and/or Informed Consent

All applicable institutional, national and international guidelines for the care and use of animals were followed.

Results

Family Physalopteridae Railliet, 1893

***Heliconema monopteri* n. sp.**
(Figs. 1, 2)
Description: Medium sized, whitish nematodes with thick, transversely striated cuticle. Cephalic end rounded. Cuticle in cephalic region inflated to form cephalic vesicle extending posteriorly to about level of deirids and anteriorly forming somewhat extended collar (Figs. 1A–C, 2C). Oral aperture dorsoventrally elongate, oval, rather large, surrounded by 2 massive, rounded lateral pseudolabia. Each pseudolabium bears 2 large submedian (dorsolateral and ventrolateral) cephalic papillae and oval laterotermi nal depression filled with irregularly lobular mass; small lateral amphids situated between both cephalic papillae (Figs. 1C, 2A–C). Inner surface of each pseudolabium with elongate lateral mound bearing marked triangular terminal lateral tooth (internolateral tooth) situated immediately near inner border of cephalic depression and simple flat tooth at each dorsoventral extremity; no denticles present near terminal lateral teeth (Figs. 1C, 2A–C). Buccal cavity short. Oesophagus divided into short, narrow anterior muscular portion and much longer, wide glandular portion. Nerve ring encircles muscular oesophagus approximately at its middle or somewhat posterior to it. Small simple deirids situated at about level of nerve ring (Fig. 1B). Excretory pore slightly anterior to anterior end of glandular oesophagus (Fig. 1A). Tail of both sexes with rounded tip.
Male (3 specimens; measurements of holotype in parentheses): Length of body 23.39 – 27.35 (23.39) mm, maximum width 340 – 394 (340). Pseudolabia 24 – 27 (24) long. Cephalic vesicle 286 – 299 (286) long and 258 – 272 (272) wide. Buccal cavity 24 – 27 (27) long. Entire oesophagus 3.48 – 3.55 (3.48) mm long, representing 11 – 15 (15)% of body length; muscular oesophagus 449 – 490 (490) long and 82 – 95 (82) wide; glandular oesophagus 2.99 – 3.10 (3.10) mm long and 163 – 177 (163) wide; length ratio of two parts of oesophagus 1:6.1 – 6.9 (1:6.1). Nerve ring, deirids and excretory pore 313 – 354 (326), 299 – 313 (313) and 420 – 530 (420) from anterior extremity, respectively. Caudal end spirally coiled, provided with lateral alae supported by 4 pairs of subventral pedunculate preanal papillae arranged in couples, and 5 single pairs of subventral postanal papillae, which are rather large and pedunculate; an additional pair of small postanal sessile papillae situated ventrally slightly posterior to level of last subventral postanal pair (Figs. 1E,G, 2E). Pair of minute phasmids present posterior to ventral pair of posteriormost postanal papillae (Fig. 1E,G). Ventral surface between posteriormost ventral postanal papillae and phasmids elevated to form distinct small protuberance. Cloacal lips somewhat elevated (Fig. 2E). Ventral precloacal surface with about 12 longitudinal tesselated ridges (area rugosa) (Figs. 1E, 2D,E). Spicules unequal and dissimilar; left spicule 465 – 510 (468) long, with sharply pointed tip; right spicule broader, boat-shaped, 186 – 225 (186) long, tapered towards distal tip. Length ratio of spicules 1:2.07 – 2.62 (1:2.52). Length of tail 340 – 367 (340).
Female (7 ovigerous specimens; measurements of allotype in parentheses): Length of body 29.29 – 34.07 (34.07) mm, maximum width 517 – 571 (571). Pseudolabia 27 – 41 (41) long. Cephalic vesicle 272 – 340 (272) long and 258 – 326 (272) wide. Buccal cavity 27 – 36 (36) long, 27 – 36 (36) wide. Entire oesophagus 3.80 – 4.62 (4.58) mm long, representing 11 – 17 (13)% of body length; muscular oesophagus 490 – 585 (571) long and 95 (95) wide; glandular oesophagus 3.31 – 4.04 (4.01) mm long and 150 – 190 (190) wide; length ratio of two parts of oesophagus 1:1.6 – 7.0 (1:7.0). Nerve ring, deirids and excretory pore 313 – 381 (340), 299 – 367 (354) and 510 – 544 (535) from anterior extremity, respectively. Vulva postequatorial, situated 16.32 – 20.03 (20.03) mm from anterior end of body, at 54 – 66 (59)% of body length. Vulval lips not elevated. Vagina narrow, muscular, directed posteriorly from vulva. Uteri containing numerous oval, thick-shelled, embryonated (larvated) eggs (Fig. 1F); eggs 39 – 45 × 27 – 30 (42 – 45 × 27 – 30), with wall 4 – 5 (4 – 5) thick. Tail short, 177 – 190 (190), with rounded tip; pair of small lateral phasmids situated near tail tip (Fig. 1D).

Taxonomic summary
Type host: Cuchia, *Monopterus cuchia* (Hamilton) (Synbranchidae, Synbranchiformes).
Site of infection: Stomach and intestine.
Type locality: Fish market in Bijnor (fish allegedly caught in the River Ganga), Bijnor district (29.3724°N, 78.1358°E), Uttar Pradesh, India (collected in August 2018).
Prevalence and intensity: 2 fish infected/2 fish examined; 30 and 40 nematode specimens.
Type specimens: Helminthological Collection of the Institute of

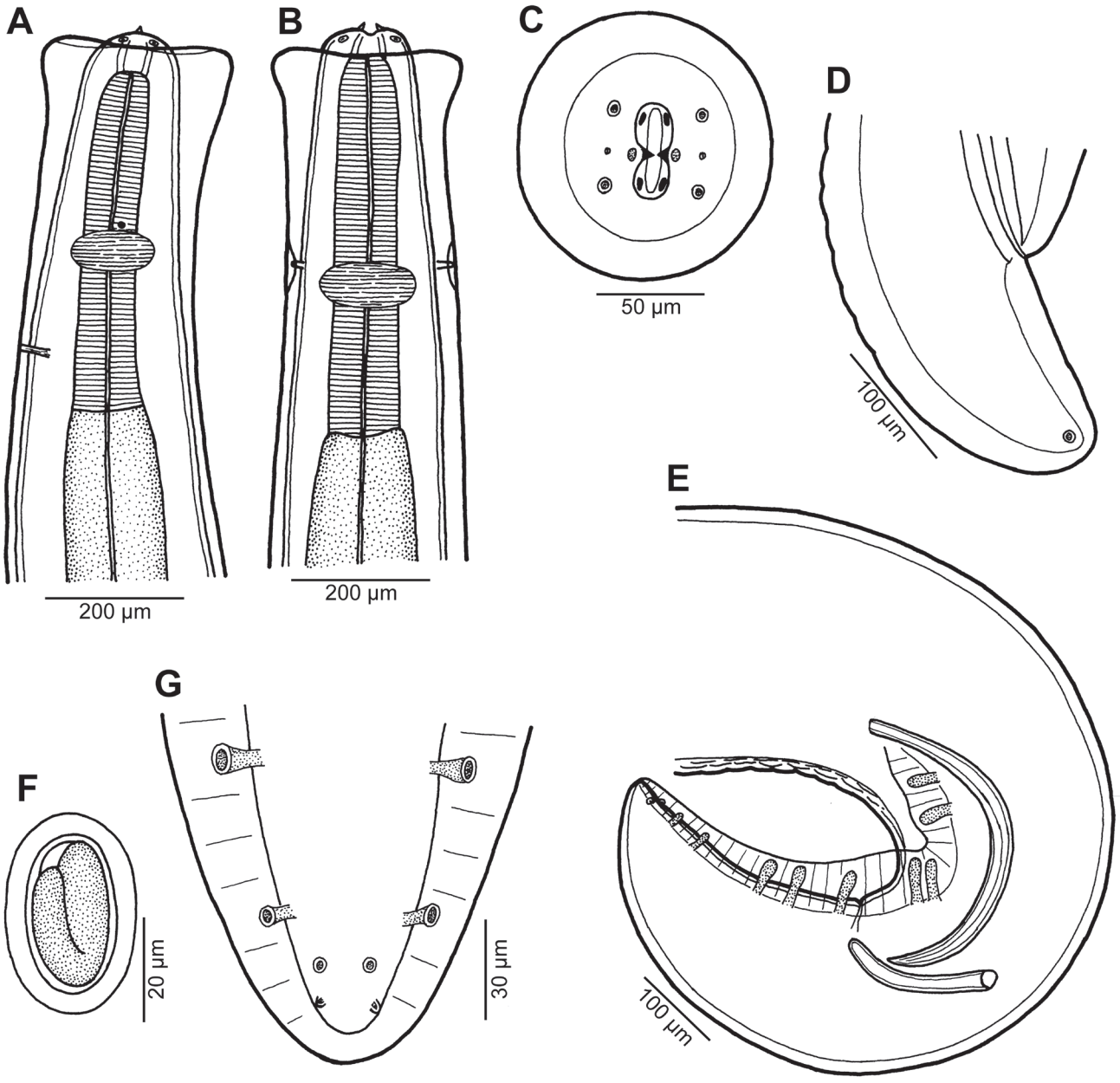

Fig. 1. *Heliconema monopteri* n. sp. A,B – anterior end, lateral and dorsoventral views, respectively; C – cephalic end, apical view; D – tail of female, lateral view; E – posterior end of male, lateral view; F – egg; G – tail tip of male, ventral view

Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic, Cat. No. N – 1186.

Etymology: The specific name of this nematode relates to the genitive form of the generic name of the host.

Discussion

The following ten recognisable species of *Heliconema* were reported by Li *et al*. (2013): *H. baylisi* Ogden, 1969, *H. brevispiculum* Baylis, 1934, *H. brooksi* Crites et Overstreet, 1991, *H. hainanense* Li, Liu, Liu et Zhang, 2013, *H. heliconema* Travassos, 1919, *H. kherai* Gupta et Duggal, 1989, *H. longissimum*, *H. psammobatidus* Threlfall et Carvajal, 1984, *H. savala* Akram, 1996 and *H. serpens* Fusco et Palmieri, 1980. However, as mentioned by Moravec and Nagasawa (2018), later this list was extended for an additional two congeneric species, *H. africanum* (Linstow, 1899) and *H. ahiri* Karve, 1941, parasites of freshwater eels (*Anguilla* spp.) in South Africa and India, respectively (Linstow, 1899; Karve, 1941; Moravec *et al*., 2013a,b).

The present taxonomy of *Heliconema* spp. is rather problemat-

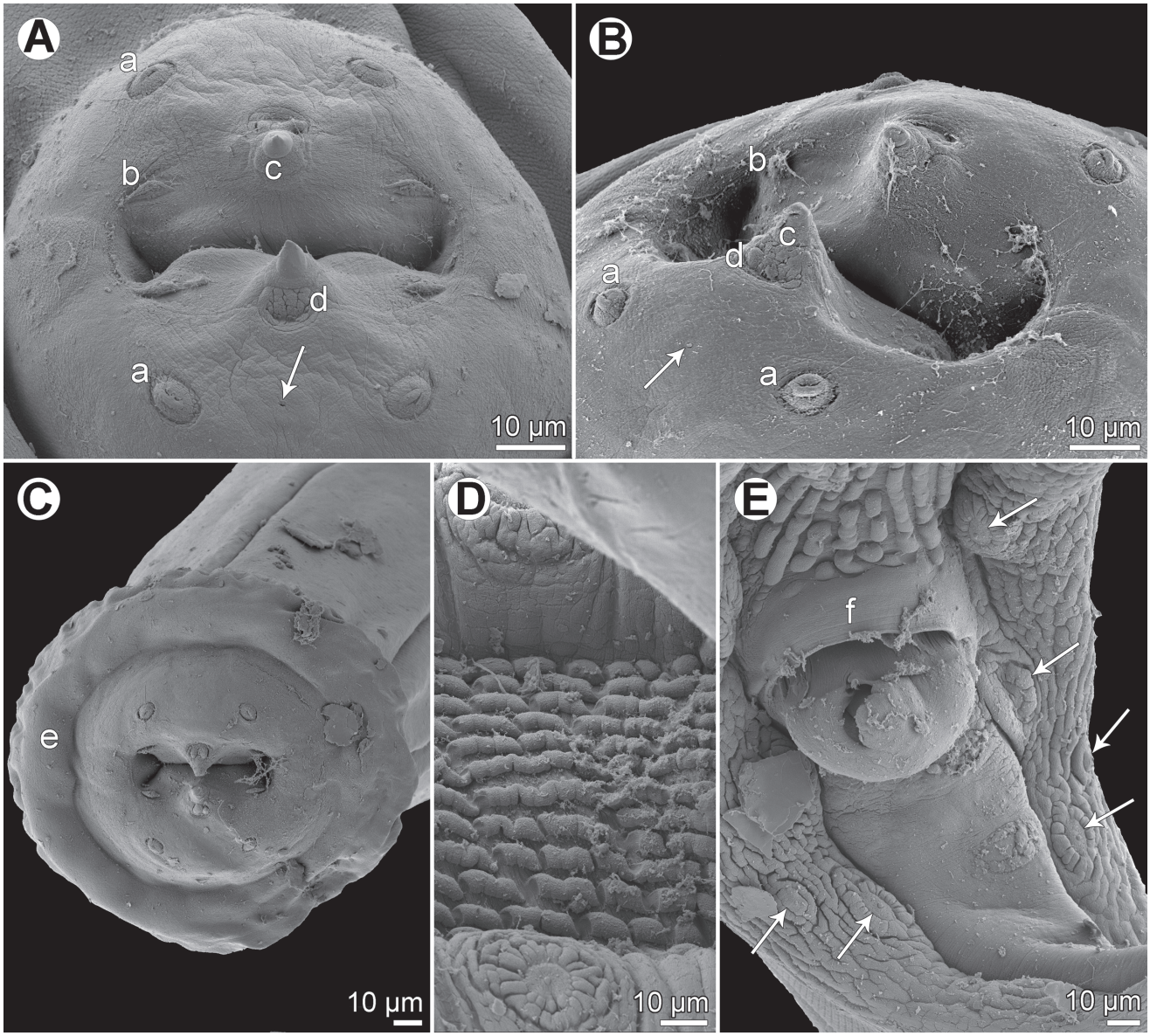

Fig. 2. *Heliconema monopteri* n. sp., scanning electron micrographs. A,B – cephalic end, two different subapical views (arrows indicate amphids); C – cephalic end, apical view; D – detail of ventral precloacal cuticular ridges (area rugosa), ventral view; E – region of cloacal aperture, ventral view (arrows indicate caudal papillae). *Abbreviations*: a – cephalic papilla; b – submedian tooth; c – lateral tooth; d – lateroapical pseudolabial depression; e – cephalic hood; f – cloacal aperture

ic, because the descriptions of older species are solely based on LM observations, whereas some important morphological features, such as the cephalic structures or the exact number and distribution of caudal papillae, require the use of SEM. The situation in *Heliconema* is further complicated by the fact that many congeneric species have similar measurements of the body and of spicules, but they can be discriminated by features difficult or impossible to observe by LM. Apparently, this often led to wrong species identifications.

Such an example is the species *H. longissimum*, originally inadequatly described as *Physaloptera longissima* by Ortlepp (1922) from snakes in Australia. Later it was redescribed from poorly preserved paratype specimens by Ogden (1969), who had also identified the nematodes at his disposal from *Mastacembelus armatus* (Lacépède) in India and those from *Anguilla mossambica* (Peters) in South Africa as *H. longissimum*; he also considered *H. anguillae* to be conspecific with *H. longissimum.* Subsequently, De *et al*. (1978) and De (1988) designated *Paraleptus komiyai* Sood, 1970 and *Notopteroides alatae* Majumdar, 1965, both parasites of *M. armatus* in India, as junior synonyms of *H. longissimum* and Moravec *et al*. (2007) synonymized *H. ahiri*, described from *Anguilla bengalensis* (Gray) in India, with this species.

However, the subsequent study of Moravec *et al*. (2013b) showed that the records of *H. longissimum* from *Anguilla mossambica* reported by Ogden (1969) and Taraschewski *et al*. (2005) in South Africa concerned in fact *H. africanum* and that the insufficiently described *H. longissimum* might be its junior synonym. Until *H. longissimum* is redescribed in detail (including the use of SEM) based on a newly collected topotypic material and the validity of this species is confirmed, this name should only be used for the type specimens originally studied by Ortlepp (1922). Chabaud and Campana-Rouget (1956) suggested that the host (unidentified Australian snakes) of Ortlepp's type material of the species is doubtful, but, according to Moravec *et al*. (2013b), apparently the snakes served only as postcyclic hosts for this parasite, which acquired the infection by feeding on its true definitive hosts (fish); some species of sea snakes are known to be specialised to feed on eels. Based on newly collected materials, *H. ahiri* and *H. anguillae* were resurrected by Moravec *et al*. (2013a) and Katahira and Nagasawa (2015), respectively. Accordingly, the nematodes reported as *H. longissimum* from *Anguilla japonica* Temminck et Schlegel in China by Li (1934) and others (see Li *et al*., 2013) belonged to *H. anguillae*.

Ogden (1969), De *et al*. (1978) and De (1988) reported *H. longissimum* from *Mastacembelus armatus* in India; De *et al*. (1978) and De (1988) considered *Paraleptus komiyai* and *Notopteroides alatae*, respectively, parasites of the same host species in the same region, to be synonyms of *H. longissimum* (see above). Later, Gupta and Duggal (1989) described *H. kherai* Gupta et Duggal, 1989 from *M. armatus* in India. Because of the morphological similarity of all these Indian forms, occurring in the same host species (*M. armatus*) that, in contrast to hosts of other *Heliconema* spp., belongs to the fish family Mastacembelidae, we consider them to represent one and the same species, *H. kherai*. Unfortunately, the original description of *H. kherai* is inadequate, but conspecific nematodes (as *H. longissimum*) were relatively well described by other above-mentioned authors. The species has not yet been examined by SEM.

Moravec *et al*. (2007) described *Heliconema* specimens from the rice-paddy eel *Pisodonophis boro* (Ophichthidae) in Thailand and identified them as *H. longissimum*. However, due to an insufficiently known morphology of *H. longissimum*, the validity of this species is uncertain (see above). Moravec *et al*. (2007) reported markedly large eggs (as compared with other *Heliconema* spp.) in the Thai specimens from *P. boro*, which was questioned by Katahira and Nagasawa (2015). However, the recent re-examination of these specimens deposited in the Helminthological Collection of the Institute of Parasitology, Czech Academy of Sciences surprisingly revealed that their uteri were largely filled with very numerous free first-stage larvae and small, non-shelled developing eggs, whereas typical embryonated, shelled eggs were quite rare. The presence of free larvae instead of shelled eggs is a unique feature among all hitherto known representatives of *Heliconema* as well as all physalopterid nematodes, in which only shelled eggs have been described. Considering also the type of the host (a representative of the Ophichthidae), we propose to establish a new species for these Thai specimens from *P. boro*, based on the description provided by Moravec et al. (2007); we propose the name ***H. pisonodophidis* n. sp.** for this species (syntypes deposited in the Helminthological Collection, Institute of Parasitology CAS, České Budějovice, Czech Republic; Cat. No. N – 862). The distinction of *H. pisodonophidis* from other congeners is apparent from the key at the end of Discussion.

By the general morphology, the new species *H. monopteri* n. sp. is most similar to *H. africanum*, *H. anguillae* and *H. pisodonophidi*s n. sp., differing from them mainly in having a shorter left spicule. Differences from all species of *Heliconema* are apparent from the key at the end of Discussion.

To date, six nominal species of *Heliconema* have been reported from South Asia (Bangladesh, India and Pakistan) (see also Sood, 2017): *H. ahiri* from *Anguilla bicolor* McClelland and *A. bengalensis* in India (Karve, 1941; Moravec *et al*., 2013a), *H. brevispiculum* from *Channa marulius* (Hamilton) in Bangladesh (Khan and Yaseen, 1969), *H. hamiltonii* Bilqees et Khanum, 1970 from *Sicamugil hamiltonii* (Day) in Pakistan (Bilqees and Khanum, 1970), *H. heliconema* from *Muraenesox cinereus* (Forsskål) in India and Pakistan (Khan and Begum, 1971; Gupta and Garg, 1976), *H. kherai* from *Mastacembelus armatus* in India (De *et al*., 1978; De, 1988; Gupta and Duggal, 1989) and *H. savala* from *Lepturacanthus savala* (Cuvier) in Pakistan (Akram, 1996); nematodes of the seventh species, *H. longissimum*, reported from India, are now considered to belong to *H. kherai* (see above).

However, except for *H. ahiri* and *H. kherai*, the congeneric nematodes from South Asia were poorly described and, judging from their unusual hosts, their species or generic identification seems to be doubtful. In particular this concerns females allegedly of *H. brevispiculum* reported from a freshwater fish (*C. marulius*) or the female nematodes described as a new species *H. hamiltonii*; the latter species was evidently based on anisakid larvae (!), as visible from illustrations, and it should be designated a *species dubia*. Consequently, *H. monopteri* n. sp. represents an additional species of *Heliconema* in South Asia with well-known morphology and the second species in this region studied by SEM.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

We thank the head of the Department of Zoology, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India, for providing laboratory facilities. Thanks are also due to the Laboratory of Electron Microscopy, Institute of Parasitology, Biology Centre CAS, institution supported by the MEYS CR (LM2015062 Czech-BioImaging) for their support with obtaining scientific data presented in this

Key to species of *Heliconema*:

1 Left spicule more than 2 mm long 2
- Left spicule less than 1 mm long 4

2 Adanal papillae absent; left spicule 2.09 – 3.50 mm long. Parasitic in Ophichthidae (*Ophichthus*); Gulf of Mexico ..... *H. brooksi*
- Adanal papillae 1 – 2 pairs; left spicule equal or longer than 3.5 mm 3

3 Three pairs preanal papillae, 2 pairs adanal papillae; left spicule 4 mm long, right spicule 270 µm; vulva at approximately first third of body. Parasitic in Trichiuridae (*Lepturacanthus*); off Pakistan ..... *H. savala*
- Four pairs preanal papillae, 1 pair adanal papillae; left spicule 3.5 – 7.3 mm, right spicule 300 – 350 µm; vulva at approximately second quarter of body. Parasitic in Muraenidae (*Echidna*) and Muraenesocidae (*Muraenesox*); Atlantic and Indian Oceans (off Trinidad, Senegal and Pakistan) ..... *H. heliconema*

4 Pair of ventral postanal papillae just posterior to cloacal aperture present; pseudolabial lateroterminal depression present 5
- Pair of ventral postanal papillae just posterior to cloacal aperture absent; pseudolabial lateroterminal depression present or absent 6

5 Postanal papillae of fifth subventral pair pedunculate; vulva preequatorial; size of eggs 39 – 51 × 20 – 30 µm; length of left and right spicule 538 – 820 µm and 200 – 306 µm, respectively. Parasitic in Anguillidae (*Anguilla*); East Asia (Japan, China) ..... *H. anguillae*
- Postanal papillae of fifth subventral pair sessile; vulva preequatorial to somewhat postequatorial; eggs 75 – 81 × 42 – 48 µm, but uterus mostly filled with free larvae; length of left and right spicule 520–734 µm and 232 – 286 µm, respectively. Parasitic in Ophichthidae (*Pisodonophis*); South-East Asia (Thailand) .... *H. pisodonophidis* n. sp.

6 Vulva distinctly postequatorial; pseudolabial lateroterminal depression present; dorsoventral flat teeth on each pseudolabium without nearby denticles; length of left and right spicule 468 – 510 µm and 186 – 225 µm, respectively. Parasitic in Synbranchidae (*Monopterus*); South Asia (India) ..... *H. monopteri* n. sp.
- Vulva equatorial, exceptionally may be slightly postequatorial; pseudolabial lateroterminal depression present, absent or not reported; dorsoventral flat teeth on each pseudolabium with or without nearby denticles 7

7 Dorsoventral flat teeth on each pseudolabium with nearby denticles; pseudolabial lateroterminal depressions present; length of left and right spicule 408 – 770 µm and 168 – 270 µm, respectively. Parasitic in Anguillidae (*Anguilla*); South Asia (India) ..... *H. ahiri*
- Dorsoventral flat teeth on each pseudolabium without nearby denticles 8

8 Pseudolabial lateroterminal depressions present; vulva preequatorial; length of left and right spicule 650 – 857 µm and 290 – 400 µm, respectively. Parasitic in Anguillidae (*Anguillla*); South Africa ..... *H. africanum*
- Pseudolabial lateroterminal depressions absent or not reported; vulva equatorial or postequatorial. Parasitic in other host families 9

9 Parasites of teleosts or elasmobranchs 10
- Parasites reported from snakes 14

10 Pseudolabial lateroterminal depressions absent; vulva preequatorial; length of left and right spicule 420 – 630 µm and 190 – 300 µm, respectively. Parasitic in Congridae (*Uroconger*) and Muraenidae (*Congresox*, *Muraenesox*); South China Sea..... *H. hainanense*
- Pseudolabial lateroterminal depressions not reported 11

11 Parasites of Anguilliformes or Synbranchiformes ……..……..……..…….. 12
- Parasites of Rajiformes: Vulva equatorial, with elevated anterior lip; length of left and right spicule 558 – 621 µm and 131 – 151 µm, respectively. Parasitic in Arhynchobatidae (*Sympterigia*); off Chile ……
……..……..…… *H. psammobatidus*

12 Parasites of Synbranchiformes: Vulva equatorial to slightly postequatorial, with non- elevated anterior lip; length of left and right spicule 420 – 630 µm and 190 – 300 µm, respectively. Parasitic in Mastacembelidae (*Mastacembelus*); South Asia (India) ……..……..……..…… *H. kherai*
- Parasites of Anguilliformes ……..……..…… 13

13 Length of left and right spicule 380 – 440 µm and 280 – 380 µm, respectively. Parasitic in Muraenidae (*Echidna*); Indian Ocean ……..……..…….. *H. baylisi*
- Length of left and right spicule 500 – 520 µm and 230 – 250 µm, respectively; vulva equatorial with elevated anterior lip. Parasitic in Muraenesocidae (*Muraenesox*); off Australia ……..……..…… *H. brevispiculum*

14 Vulva preequatorial; length of left and right spicule 516 µm and 228 – 300 µm, respectively; size of eggs 59 × 32 µm. Reported from unidentified snakes; Australia ……..……..…… *H. longissimum*
- Vulva situated in about mid-length of body, anterior vulval lip non-elevated; length of left and right spicule 404 – 525 µm and 247 – 314 µm, respectively; size of eggs 49 – 54 × 27 – 31 µm. Parasitic in Homalopsidae (*Cerberus*); Malaysia ……..…… *H. serpens*

paper, and to Blanka Škoríková of the same Institute for help with illustrations. This study was partly supported by the institutional support of the Institute of Parasitology, BC AS CR, 585110/9500).

References

Akram M.C. (1996): *Heliconema savala* n. sp. (Nematoda: Physalopteridae) from marine fish *Lepturacanthus savala* (Cuvier) (Trichuridae) and *Psettodes erumei* (Schneider) new host (Pisces: Psettotidae) of physalopterid nematode, *Bulbocephalus inglisi* Rasheed, 1966 in Pakistan. *Pakistan J. Mar. Sci*., 5: 132 – 143

Bilqees F.M., Khanum Z. (1970): Marine fish nematodes of West Pakistan II. A new species of the genus *Heliconema* from fishes of Pasni. *Pakistan J. Zool*., 2: 211 – 213

Chabaud A.G., Campana-Rouget Y. (1956): Le genre *Ortleppina* Schulz, 1927, parasite d'Apodes, et non de Serpents, est synonyme de genre *Heliconema* Travassos, 1919. *Ann. Parasitol. Hum. Comp*., 31: 308 – 309

De N.C. (1988): Remarks on the validity of the species *Notopteroides alatae* Majumdar, 1965. *Folia Parasitol*., 35: 281 – 284

De N.C., Ghosh M., Majumdar G. (1978): Records of some little known nematodes from Indian fishes. *Folia Parasitol*., 25: 317 – 322

Froese R., Pauly D. (Eds) (2018): *FishBase*. World Wide Web electronic publication. http://www.fishbase.org, version 12/2018

Gupta N.K., Duggal C.L. (1989): On a new species of the genus *Heliconema* (Nematoda: Physalopteridae) from a freshwater fish with a key to the species of the genus. *Res. Bull*. (*Sci*.) *Punjab Univ*., 40: 73 – 75

Gupta N.K., Garg V.K. (1976): Observations on three already known spiruroid nematodes from marine food fishes of South India. *Indian J. Helminthol*., 28: 17 – 28

Karve J.N. (1941): Some parasitic nematodes of fishes. I. *J. Univ. Bombay*, *New Ser*., 10B: 9 – 42

Katahira H., Nagasawa K. (2015): *Heliconema anguillae* Yamaguti, 1935, a physalopterid nematode found in Japanese eels: taxonomic resurrection with a note on the third-stage larva from intertidal crabs in western Japan. *Folia Parasitol*., 62: 028. DOI: 10.14411/fp.2015.028

Khan D., Begum A. (1971): Helminth parasites of fishes from West Pakistan I. Nematodes. *Bull. Dept. Zool*., *Univ. Punjab* (*New Ser*.), 5: 1 – 22

Khan D., Yaseen T. (1969): Helminth parasites of fishes from East Pakistan I. Nematodes. *Bull. Dept. Zool*., *Univ. Punjab* (*New Ser*.), 4: 1 – 33

Li H.C. (1934): Report on a collection of parasitic nematodes, mainly from North China. Part II. Spiruroidea. *Trans. Am. Microsc. Soc*., 53: 174 – 195

Li L., Liu Y.-Y., Liu B.-C., Zhang L.-P. (2013): Morphological and molecular characterization of *Heliconema hainanensis* sp. nov. (Spirurina: Physalopteridae) from congers in the South China Sea, with a key to the species of *Heliconema*. *Mem. Inst. Oswaldo Cruz*, 108: 41 – 47. DOI: 10.1590/s0074-02762013000100007

Linstow, O., von (1899): Nematoden aus der Berliner Zoologischen Sammlung. *Mitt. Zool. Sam. Mus. Naturk. Berlin*, 1: 3 – 28, 6 Plts

Moravec F., Nagasawa K. (2018): *Rhabdochona angusticaudata* sp. n. (Nematoda: Rhabdochonidae) from the Japanese eel *Anguilla japonica*, and new records of some other nematodes from inland fishes in Japan. *Folia Parasitol*., 65: 016. DOI: 10.14411/fp.2018.015

Moravec F., Sheeba S., Kumar A.B. (2013a): Observations on nematodes from the Indonesian shortfin eel *Anguilla bicolor bicolor*

McClelland in India, including a revalidation of *Heliconema ahiri* Karve, 1941 (Physalopteridae). *Acta Parasitol*., 58: 496 – 503. DOI: 10.2478/s11686-013-0168-0
Moravec F., Taraschewski H., Anantaphruti M.T., Maipanich W., Laoprasert T. (2007): *Heliconema longissimum* (Ortlepp, 1923) (Nematoda: Physalopteridae) from *Pisodonophis boro* (Teleostei: Ophichthidae) in Thailand, with remarks on the taxonomy of the Proleptinae Schulz, 1927. *Syst*. *Parasitol*., 66: 73 – 80. DOI: 10.1007/s11230-006-9066-2
Moravec F., Taraschewski H., Weyl O.L.F. (2013b): Redescription of *Heliconema africanum* (Linstow, 1899) n. comb. (Nematoda: Physalopteridae), a nematode parasite of freshwater eels (*Anguilla* spp.) in South Africa. *Syst*. *Parasitol*., 85: 263 – 269. DOI: 10.1007/s11230-013-9421-z
Ogden C.G. (1969): A revision of the genus *Heliconema* Travassos, 1919, Physalopteridae (Nematoda). *J*. *Nat*. *Hist*., 3: 423 – 431
Ortlepp R.J. (1922): The nematode genus *Physaloptera* Rud. *Proc*. *Zool*. *Soc*. *Lond*., 1922: 999 – 1107
Sood M.L. (2017): *Fish nematodes from South Asia*. Second edition. Kalyani Publishers, Ludhiana, 1039 pp
Taraschewski H., Boomker J., Knopf K., Moravec F. (2005): *Anguillicola papernai* (Nematoda: Anguillicolidae) and other helminths parasitizing the African longfin eel *Anguilla mossambica*. *Dis*. *Aquat*. *Org*., 63: 185 – 195

DOI 10.2478/helm-2019-0009

HELMINTHOLOGIA, 56, 2: 132 – 140, 2019

Metazoan parasites of common sole (*Solea solea*) and scaldfish (*Arnoglossus laterna*) (Pleuronectiformes) from Sinop coast of Black Sea

A. GÜVEN,T. ÖZTÜRK*

[1]Sinop University, Faculty of Fisheries and Aquatic Sciences, 57000 Sinop, Turkey,
E-mail: **turkay.ozturk@gmail.com*, *arzu.cam86@gmail.com*

Article info

Received October 24, 2018
Accepted February 26, 2019

Summary

The metazoan parasites were investigated of two flatfish the common sole, *Solea solea* (n:140) and the scaldfish, *Arnoglossus laterna* (n: 22) in the Sinop coast of Black Sea from June 2015 to June 2017. A total of 15 metazoan parasite species belonging to Digenea (6), Cestoda (3), Acanthocephala (1), Nematoda (4) and Isopoda (1) taxonomic groups were identified. *Solea solea* was found to be infected by *Condylocotyla pilodora*, *Proctoeces maculatus*, Opecoelidae gen. sp., *Metadena* sp., *Stephanostomum* sp., *Progrillotia* sp., *Capillaria gracilis*, *Cucullanus campanae*, *Solearhynchus rhytidotes* and *Nerocila orbignyi. Arnoglossus laterna* was found to be infected by *Lecithochirium musculus* and *Grillotia erinaceus. Scolex pleuronectis, Hysterothylacium aduncum* and *Dichelyne minutus* were determined in both flatfish. Infection prevalence and mean intensity values were recorded for each parasite species. Infection values for each parasite species in relation to season and fish size were also determined and compared as comparatively. This study is the first one assessing the metazoan parasites both of *S. solea* and *A. laterna* collected from the Turkish coast of Black Sea. While *Condylocotyle pilodora* and *Capillaria gracilis* are new parasite records for *S. solea*, *Grillotia erinecaus* is new parasite record for *A. laterna*. Moreover, this paper is the first report on occurrence of Opecoelidae gen. sp., *Metadena* and *Stephanostomum* genera in *S. solea*.
Keywords: Common sole; Scaldfish; Metazoan parasite; Sinop; Black sea

Introduction

Pleuronectiformes, also called flatfish, are characterized by oval-shaped, flattened bodies, unique among fishes in being asymmetrical with both eyes on one side in adults. Flatfish fishery is among the most profitable demersal types of activity, since these species are economically and ecologically important part of benthic ecosystems in the world (Diaz de Astarloa, 2002; Gibson, 2005). The common sole, *Solea solea* (Linnaeus, 1758) and the scaldfish, *Arnoglossus laterna* (Walbaum, 1792) are flatfish inhabiting sandy or muddy marine bottoms of the sea floor. They feed on small bottom-dwelling invertebrates, including polychate, bivalves and crustaceans. They are to be found in the Black Sea and in the Mediterranean Sea along the west coast of Europe to Norway (Nielsen, 1973). *A. laterna* is caught in commercial trawlers, but it is usually discarded because of this fish species generally not being preferred for human consumption. Although it is not a commercially important species, it is important ecologically as it serves within the food chain for other predator fish. However, *S. solea* is a species with high commercial interest, moreover it has been shown as suitable species for aquaculture (Fanelli, 2008).
Parasites are a natural part of all ecosystems and they represent important component of the biodiversity of shallow coastal areas (Combes, 2001). Besides they play an important role for their host,

* – corresponding author

as they give information about both physiology and ecology (etc. growth, condition, survival, fecundity, behaviour and population structure) of their hosts (Combes, 2001; MacKenzie and Abaunza, 2005). Parasitism is an important factor that could substantially affect on host biology, ecology and phylogeny (Marques *et al*., 2011). In reared fish species, it is extremely important to know which parasites are found in the target fish species. The aim of present study was to reveal the metazoan parasites of two flatfish, *S. solea* and *A. laterna* in Sinop region of the Black Sea. The diversity of parasites in relation to seasonal and size classes of the two flatfish were investigated and described for the first time in the Turkish coast of the Black Sea, in order to contribute to the knowledge on parasitofauna of demersal fish healh.

Materials and Methods

Fish samples were collected from the southern Black Sea off Sinop (N 42° 01' 55" E 35° 16' 36") (Fig. 1). A total of 162 flatfish; common sole *S. solea* (n:140), 22 and scaldfish *A. laterna* (n:22) were collected between June 2015 – June 2017 with gill nets and beach seine. Fish were transferred to the laboratory and some part of them examined freshly and some part of them frozen to be examined later. At necropsy, total length and total weight of fish were mesaured. Fish were grouped in three size classes after total lengths has been measured: class 1: <14.0 cm, class 2: 14.0-15.9 cm, class 3: >15.9 cm. The flatfish were dissected to check for metazoan parasites using conventional methods; assessing smears of skin, fins, gills, eyes (lens and vitreous humour), kidney, liver, stomach and intestine of fish under the dissecting microscope and then counting parasite specimens. Parasite species were identified by Gibson, 2002, Bray, 2005a;b, Miller and Cribb, 2008, Pearson, 2008 for digeneans, Lebre and Petter, 1984, Køie, 1993, Koie, 2001a, Arai and Smith, 2016 for nematodes, Campbell and Beveridge, 1994, Beveridge *et al*., 2004, Beveridge and Campbell, 2007 for cestodes, Belofastova, 2006 for Acanthocephala and Al-Zubaidy and Mhaisen, 2013 for Isopoda. All parasite species were examined using a stereo Olympus microscope (BX53) equipped with a digital camera (DP50). The terms prevalence (percentage of fish infected), mean intensity (mean number of parasites per infected fish), and mean abundance (mean number of parasites per examined fish) were calculated following the definitions of Bush *et al*. (1997). The significance of the host-parasite relationship was determined according to the abundance values; core species were those represented by >2 parasite species, secondary species by 0.6-2, satellite species by 0.2 – 0.6 and rare species 0.2 (Zander, 2003). The mean infracommunity was characterized as the mean number of parasite species per host individual (Zander, 2004). The differences in the mean intensities values of each parasite species for length classes of fish and seasons were tested by the Kruskal-Wallis test (Nonparametric ANOVA). All statistical analysis were done using Graph Pad Instat Software. Statistical significance level was evaluated at 0.05.

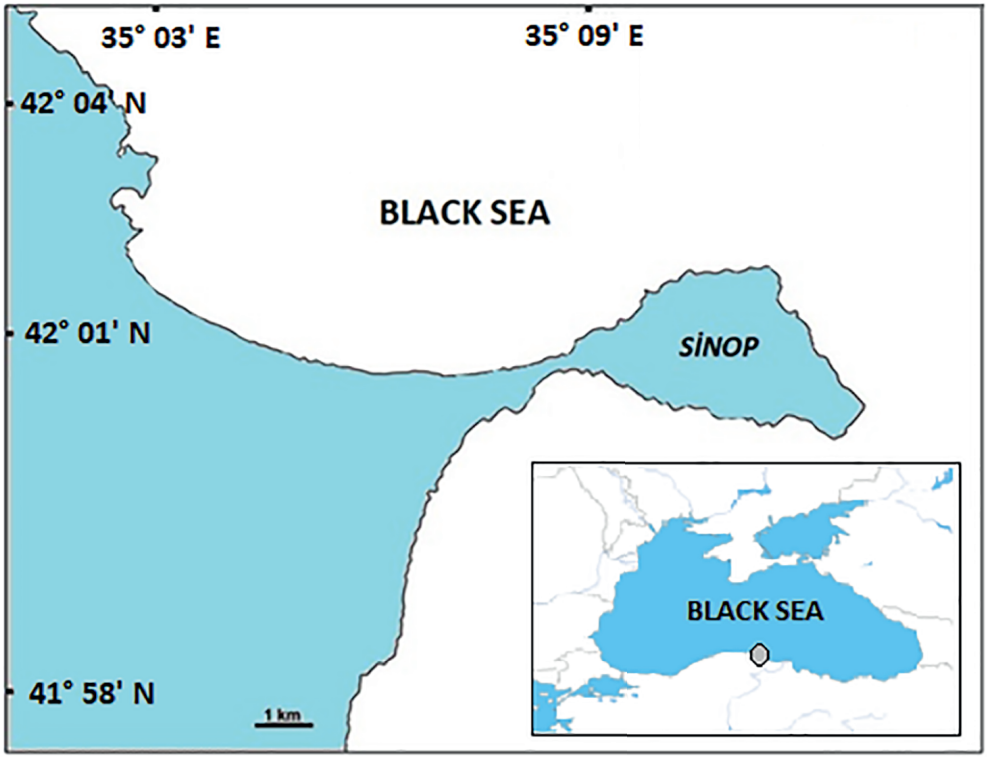

Fig. 1. The southern Black Sea off Sinop.

Table 1. Parasite list with indications of infection site (microhabitat), prevalence (%), mean intensity (MI) and abuncance (A) in both flatfish host.

	Solea solea (n: 140)				*Arnoglossus laterna* (n: 22)			
Parasites	**Infection Site**	**P (%)**	**MI**	**A**	**Infection Site**	**P (%)**	**MI**	**A**
Digenea								
Lecithochirium musculus					intestine, stomach	81.82	26.39	**21.59**
Proctoeces maculatus	intestine	2.14	1.00	0.02				
Condylocotyla pilodora met.	brain	2.14	2.00	0.04				
Opecoelidae gen. sp. met.	gill	18.57	3.00	0.56				
Stephanostomum sp. met.	gill	4.29	1.33	0.06				
Metadena sp. met	gill, eye, kidney	23.57	10.43	**2.47**				
Cestoda								
Scolex pleuronectis pl.	intestine	5.71	18.62	1.06	intestine	81.82	85.72	**70.14**
Grillotia erinaceus					intestine	77.27	15.65	**12.09**
Progrillotia sp.	intestine, gall bladder	6.43	5.44	0.35				
Nematoda								
Hysterothylacium aduncum	intestine	3.57	2.40	0.09	intestine	45.45	5.10	**2.32**
Capillaria gracilis	intestine,	37.86	3.57	1.35				
Cucullanus campanae	intestine	43.57	5.36	**2.34**				
Dichelyne minutus	intestine	72.14	26.09	**19.05**	intestine	9.10	5.00	0.45
Acanthocephala								
Solearhynchus rhytidotes	intestine	28.57	3.67	0.81				
Isopoda								
Nerocila orbignyi	Fins, skin	3.57	2.00	0.31	-	-	-	-
Overall		81.43	26.70[a]	21.74[a]		100	106.59[b]	106.59[b]

Ethical Approval and/or Informed Consent

The research related to animals use has been conformed with all the particular national regulations and institutional policies for the care and use of animals. The study protocol no. 19 of 13/07/2015 was approved by Republic of Turkey, Sinop University Experimental Animals Local Ethics Committee.

Results

In this study a total of 15 metazoan parasites were identified: five species of Digenea; *Lecithochirium musculus* (Hemiuridae), *Proctoeces maculatus* (Fellodistomidae), *Condylocotyla pilodora* met. (Heterophyidae), Opecoelidae gen. sp. met. (Opecoelidae), *Stephanostomum* sp. met. (Acanthocolpidae), *Metadena* sp. met. (Cryptogonimidae), three of cestodes; *Scolex pleuronectis* pl. (Tetraphyllidea incertae sedis), *Grillotia erinaceus* (Lacistorhynchidae), *Progrillotia* sp. (*Progrillotiidae*) four of Nematoda; *Hysterothylacium aduncum* (Raphidascarididae), *Capillaria gracilis* (Capillariidae), *Cucullanus campanae* and *Dichelyne minutus* (Cucullanidae), one of Acanthocephala; *Solearhynchus rhytidotes* (Echinorhynchidae) and one of Isopoda; *Nerocila orbignyi* (Cymothoidae). Table 1 summarises parasite list with indications of infection site (microhabitat), prevalence (%), mean intensity (MI) and abuncance (A) in both flatfish host. While the common sole was infected with 13 parasite species, the scaldfish was infected with only 5 parasite species. The cestode *Scolex pleuronectis*, the nematodes *Hysterothylacium aduncum* and *Dichelyne minutus* were determined in both host. *Lecithochirium musculus* and *Grillotia erinaceus* were found only in the scaldfish; *Proctoeces maculatus*, *Condylocotyla pilodora*, Opecoelidae gen. sp. met., *Metadena* sp., *Stephanostomum* sp., *Progrillotia* sp., *Capillaria gracilis*, *Cucullanus campanae*, *Solearhynchus rhytidotes* and *Nerocila orbignyi* were found only in the common sole (Table 1). Seven species, *L. musculus*, *Metadena* sp., *S. pleuronectis*, *G. erinaceus*, *H. aduncum*, *C. campanae* and *D. minutus* are designated as core species in the metazoan parasite faunas of two flatfish. In the common sole, the dominant species was *D. minutus*, while in the scaldfish, it was *S. pleuronectis* (Table 1).

Seasonal prevalence and mean intensities of metazoan parasite species in flatfish are showed in Table 2. The species composition and the parasite species richness of the two flatfish were found to be different from each other and varied among the seasons. The overall mean intensity values of parasite species on the common sole varied significantly among the seasons ($P<0.05$) and the highest mean intensity occurred in spring. *D. minutus* and *S. rhytidotes* were detected in all seasons and maximum infection prevalence was 90.2 % in spring for *D. minutus* and 45.5 % in autumn for *S rhytidotes*. Metazoan parasite diversity differed between seasons. When evaluating the diversity of the parasite species depending on the seasons in the common sole, maximum value was found in spring encompassing thirteen parasite species. For the scaldfish, the parasite species richness showed no difference among seasons, except for summer. However, overall infection parameters were similar in all seasons except for summer in the scaldfish (Table 2).

The infection parameters of parasite species in selected three size classes of flatfish are given in Table 3. Opecoelidae gen. sp. met. and *Stephanostomum* sp. were absent in the smallest (<14.0 cm) and *P. maculatus* and *C. pilodora* were found only in the largest class (>15.9 cm) of common sole. The largest fish length class had higher overall mean intensities than the other length classes in the common sole, showing statistically significant difference ($P<0.05$). In the scaldfish, species richness, the overall mean intensity and prevalence values were found to be independent from fish length and showed no significant differences (Table 3). A similar pattern was also seen in the mean infracommunity values between the size classes. The minimum number of parasite species was found in the smallest size class (<14.9 cm) in the common sole (Table 3).

Discussion

This study is the first on metazoan parasites of scaldfish from Turkish coast of Black Sea and *G. erinaceus* and *D. minutus* are first geographical report for scaldfish in the Black Sea. There are few studies on metazoan parasite fauna of common sole in Turkey, it is also very limited especially in Turkish coast of Black Sea. To date, only ten metazoan parasite species have been reported in the common sole in Turkish waters according to data from different authors. These are; one species of Isopoda, *Nerocila orbigyni* (Kayış and Ceylan, 2011) and one species of Myxozoa, *Sinuolinea rebae* (Özer *et. al*, 2015) in Black Sea, three species of Arthopoda, *Caligus solea* in North Eastern Mediterranean (Demirkale *et. al*, 2014), *Caligus brevicaudatus* and *Caligus apodus* in Eastern Mediterranean (Özak *et. al*, 2013), three species of Cestoda, *Bothriocephalus scorpii*, *Grillotia* sp. and *Scolex pleuronectis* in the Marmara Sea (Keser *et. al*, 2007), one species of Digenea, Hemiuridae metacercaria in the Marmara Sea (Keser *et. al*, 2007), one species of Nematoda, *Hysterothylacium aduncum* in the Marmara Sea (Keser *et. al*, 2007) and in Eastern Mediterranean Sea (Keskin *et. al*, 2015). Other studies of the metazoan parasites of common sole showed that *Hysterothylacium aduncum*, *S. pleuronectis*, *Solearhynchus rhytidotes* (syn: *S. kostylewi*) and *Nerocila orbignyi* were previously reported. We found 13 parasite species; 6 of these had already been mentioned in published studies, but so far, 7 species, *Condylocotyla pilodora, Proctoeces maculatus,* Opecoelidae gen. sp. met., *Metadena* sp., *Stephanostomum* sp., *Capillaria gracilis* and *Progrillotia* sp. haven't been reported in the common sole, and this is their first report. But, *C. pilodora*, *P. maculatus* and *Stephanostomum* sp. have been detected in low intensity and is being registered as rare species during this investigation. Therefore, we consider that their presence in the common sole is accidental and that they are parasites of other fish species in the study area.

In this study, Digenea was the most represented taxa with a total

Table 2. Seasonal prevalence and mean intensities of metazoan parasite species in flatfish.

Parasites	*Solea solea* (n: 140)								*Arnoglossus laterna* (n: 22)							
	Spring (82)		Summer (38)		Autumn (9)		Winter (11)		Spring (3)		Summer (2)		Autumn (11)		Winter (3)	
	P	MI	P	MI	P	MI	P	MI	P	MI	P	MI	P	MI	P	MI
Lecithochirium musculus									100	92.7	100	23.5	84.6	13.5	33.3	1.0
Proctoeces maculatus	3.7	1.0	-	-	-	-	-	-								
Condylocotyla pilodora	3.7	2.0	-	-	-	-	-	-								
Opecoelidae gen. sp. met.	31.7	3.0	-	-	-	-	-	-								
Stephanostomum sp. met.	6.1	1.4	2.6	1.0	-	-	-	-								
Metadena sp. met	35.4	11.4	7.9	4.7	-	-	-	-								
Scolex pleuronectis pl.	8.5	14.3	-	-	-	-	9.1	49.0	66.6	138.5	-	-	92.3	84.9	100	68.3
Grillotia erinaceus									33.3	35.0	-	-	92.3	14.6	100	16.7
Progrillotia sp.	8.5	5.9	-	-	-	-	18.2	4.0								
Hysterothylacium aduncum	6.1	2.4	-	-	-	-	-	-	33.3	9.0	-	-	53.9	5.4	33.3	1.0
Capillaria gracilis	37.8	3.19	44.7	3.7	45.5	5.4	-	-								
Cucullanus campanae	43.9	5.5	65.8	5.2	-	-	-	-								
Dichelyne minutus	90.2	26.6^{a}	81.6	7.4^{b}	72.7	19.3^{a}	63.6	22.4^{a}	-	-	-	-	7.7	2.0	33.3	8.0
Solearhynchus rhytidotes	26.8	3.2	5.26	10.0	45.5	4.0	27.3	1.0								
Nerocila orbignyi	2.4	1.5	23.7	4.1	18.2	2.0	-	-								
Overall	98.8	35.9^{a}	86.8	14.9^{bc}	100	18.2^{ac}	100	24.1^{c}	100	199.7	100	23.5	100	106.3	100	88.3
Species richness	13		7		4		4		4		1		5		5	
Mean infracommunity	3.06		2.67		1.91		1.44		2.3		1.00		3.31		3.00	
Mean fish length	15.9^{a}		14.0^{bc}		15.2^{ac}		13.3^{c}		15.9		15.5		14.1		11.0	

Means followed by the same superscript letter are not significantly different.

6 species, 2 adults (*L. musculus* and *P. maculatus)* and 4 metacercariae stage (*C. pilodora,* Opecoelidae gen. sp. met.*, Stephanostomum* sp. and *Metadena* sp.). Among the Digeneans, only the *L. musculus* was detected in the scaldfish, the other 5 digenea parasites were detected in the common sole. Except *L. musculus,* other digeneans were observed in spring and summer seasons. Release of digenean cercariae from the snail host and successful transmission to the fish host is highly temperature-dependent. In this study the prevalence of digenean species in spring and summer may be explain with water temperature.

Fish size had an effect in both species diversity and mean intensity of digenean species. In general, mean intensity values were positively correlated with fish size in the common sole (Table 3). *Proctoeces maculatus, C. pilodora, Stephanostomum* sp. and Opecoelidae gen. sp. met. were absent in the smallest fish size class (<14.0 cm). The absence of these parasites can be explained by dietary differences related to fish length groups. Studies of dietary composition show that the food of the common sole consists of bottom-dwelling invertebrates, such as Polichaeta, Crustacea, Mollusca. The variety of habitats, for example site type, e. g. shallow coastal areas, continental shelf, estuarine systems and the range of fish lengths account for dietary dissimilarities (Muus and Dahlström 1991; Banaru and Harmelin-Vivien, 2009; Cabral 2000).

Four nematode species, *Hystherothylacium aduncum, Capillaria gracilis, Dichelyne minutus* and *Cucullanus campanae* were determined in two flatfish during the present investigation. The common sole was found to be infected with above mentioned four nematode species, the scaldfish was infected with *H. aduncum* and *D. minutus.* Among recorded nematodes, *D. minutus* and *C. campanae* as core species, *C. gracilis* as secondary species and *H. aduncum* as rare species were determined in the common sole. Whereas in the scaldfish, the *H. aduncum* was detected as core species, and *D. minutus* as satellite species (Table 1). *Cucullanus campanae* is specific parasite of the common sole (Koie, 2000). Similarly, *D. minutus* is a typical and common parasite of demersal fishes such as flounders and gobies (Moravec, 1994; Koie, 2001a). *Dichelyne minutus* uses usually the polychaete *Hediste diversicolor* as an obligate intermediate host, and members of gobiid and flatfish are the definitive hosts of this nematode (Koie, 2001a). *H. diversicolor* has been indicated as the first intermediate host of this nematode in also Black Sea and mature individuals of *D. minutus* were reported in various species of fish (Pronkina *et al*., 2017). In the present study, seasonal changes in the mean intensity and prevalence of *D. minutus* were determined. The highest prevalence value was recorded in spring (Table 2). Similarly in spring, Pronkina *et al.* (2017) reported high prevalence from the definitive hosts in the Black Sea. The results obtained in the present study are in good agreement with Pronkina *et al*. (2017).

Capillaria gracilis which is known mainly as a parasite of gadoid fish, uses usually oligochaetes and rarely fish as the intermediate hosts. It has been reported that gobies act as obligatory intermediate hosts (Koie 2001b). The prevalence value of *C. gracilis* was higher in summer than in other seasons and it was not detected in winter (Table 2). The mean intensity values were also positively correlated with increasing fish size (Table 3). Seasonal average length of fish in winter is lower and this clearly explains its absence in winter. Thus our results indicated that the infection prevalence had a relation with host size.

Scolex pleuronectis (pleurocercoid)*, Grillotia erinaceus* and *Progrillotia* sp. were the cestod parasites determined in the present study. *S. pleuronectis* and *G. erinaceus* were the core and dominant species for the scaldfish as seen in overall infection values (Table 1). However *S. pleuronectis* has been previously reported in certain fish species in the same sampling region (Güneydağ *et al*. 2017). Similarly, *G. erinaceus* has been reported in *Merlangius merlangus* in the same sampling area (Özer *et al*., 2014). Two cestodes species are reported in the scaldfish first time with this study. Moreover, the prevalence and mean intensity values obtained were significantly higher for both cestode species compared with previous reported in the region. Therefore, we suggest that the scaldfish is the principal second intermediate host for both cestode.

Solearhynchus rhytidotes is a rare parasite of the common sole. It is redescribed and reported in the common sole captured from the Sea of Marmara by Kvach and Oğuz, 2010.

Nerocila orbignyi was detected from fin and skin in the common sole. The presence of *N. orbignyi* has been previously reported in the common sole from Black Sea by Kayış and Ceylan, 2011. In this study, detailed data related with infection values both *S. rhytidotes* and *N. orbignyi* were presented for the first time in Turkey.

Although the scaldfish had lower number of parasite species then the common sole, the highest prevalence and intensity values (100 % and 106.59) were found in scaldfish (Table 1). The results of this study showed that many parasite species prefer the common sole rather than the scaldfish. It could be due to effect of dietary preferences. The scaldfish, *Arnoglossus laterna* is a highly motile and selective predatory, it feeds on small fishes and invertebrates (Fanelli *et al*., 2009). Conversely, the common sole, *Solea solea* is sedentary predatory, it feeds upon worms, mollusks, and small crustaceans that most of them are infected with parasites (Cabral 2000).

Solea solea has different prey groups compared to *A. laterna* and this study shows that this different diet compositions may affect parasite species diversity. Although this is a factor influencing parasite load and parasite diversity, diet diversity alone cannot fully explain it. Additionally our results indicated that the number of parasite species had a relation with host size in particular *S.solea*. It can be due to the composition and diversity of the ingested prey varied with the size of predator fish and increased of the diet of larger specimens than smaller ones.

In conclusion, the data presented in this study contribute to the knowledge on metazoan parasites in two flathfish and add some valuable information on its occurrence on seasonal level and the relation with hosts size.

Table 3. The infection parameters of parasite species in selected three size classes of flatfish.

Parasites	*Solea solea* (n: 140)						*Arnoglossus laterna* (n: 22)					
	< 14.0 cm (n:30)		14.0 – 15.9 cm (n:62)		15.9 cm (n:48)		< 14.0 cm (n:11)		14.0 – 15.9 cm (n:5)		15.9 cm (n:6)	
	P	MI	P	MI	P	MI	P	MI	P	MI	P	MI
Lecithochirium musculus							63.6	8.57[a]	100.0	41.4[b]	100.0	34.7[b]
Proctoeces maculatus	-	-	-	-	6.3	1.0						
Condylocotyla pilodora met.	-	-	-	-	6.3	2.0						
Opecoelidae gen. sp. met.	-	-	8.1	1.8	43.8	3.3						
Stephanostomum sp. met.	-	-	3.2	2.0	10.4	1.2						
Metadena sp. met	3.3	1.0	11.3	3.9	52.1	12.7						
Scolex pleuronectis pl.	6.7	46.0	1.6	2.0	10.4	11.0	90.9	77.1	60.0	60.3	100.0	98.5
Grillotia erinaceus							90.9	16.3	40.0	16.5	100.0	11.7
Progrillotia sp.	10.0	14.0	1.6	10.0	10.4	1.2						
Hysterothylacium aduncum	3.3	3.0	4.8	2.7	2.1	1.0	45.5	1.8	-	-	100.0	7.0
Capillaria gracilis	30.0	1.9	40.3	3.1	39.6	5.0						
Cucullanus campanae	40.0	5.3	51.6	3.9	35.4	8.2						
Dichelyne minutus	83.3	8.15	88.7	21.4	83.3	28.2	9.1	8.0	-	-	16.7	2.0
Solearhynchus rhytidotes	16.7	6.8	27.4	1.9	20.8	4.7						
Nerocila orbignyi	6.7	4.0	14.5	3.3	4.2	3.0						
Overall	90.0	17.2[a]	98.4	24.4[b]	95.8	40.8[c]	100.0	91.9	100.0	84.2	100.0	152.2
Species richness	9		11		13		5		3		5	
Mean infracommunity	2.19		2.60		3.37		3.00		2.00		3.67	

Means followed by the same superscript letter are not significantly different.

Conflict of Interest

Authors state no conflict of interest

Acknowledgement

Authors are grateful to The Scientific and Technological Research Council (TÜBİTAK) of Turkey for its financial support in a part of this study with the project number of 215O224

References

Al-Zubaidy, A. B., Mhaisen, F. T. (2013): The first record of three cymothoid isopods from Red Sea fishes, Yemeni coastal waters. International Journal of Marine Science, 3(21): 166 – 172. DOI: 10.5376/ijms.2013.03.0021

Arai, H. P., Smith, J. W. (2016): Guide to the Parasites of Fishes of Canada Part V: Nematoda. *Zootaxa*, 4185(1): 1 – 274. DOI: 10.11646/zootaxa.4185.1.1

Banaru, D., Harmelin-Vivien, M. (2009): Trophic links and riverine effects on food webs of pelagic fish of the Northwestern Black Sea. *Mar. Freshw. Res.,* 60: 529 – 540. DOI: 10.1071/MF08005

Belofastova, I. P. (2006): *Solearhynchus rhytidotes* nov. comb. (Palaeacanthocephala: Echinorhynchidae), a parasite of soles (Soleidae). Parazitologiia, 40(1): 85 – 93

Beveridge, I., Neifar, L., Euzet, L. (2004): Review of the genus *Progrillotia* Dollfus, 1946 (Cestoda: Trypanorhyncha), with a redescription of *Progrillotia pastinacae* Dollfus, 1946 and description of *Progrillotia dasyatidis* sp. n. *Folia parasitol.*, 51(1): 33. DOI: 10.14411/fp.2004.005

Beveridge, I., Campbell, R. A. (2007): Revision of the *Grillotia erinaceus* (van Beneden, 1858) species complex (Cestoda: Trypanorhyncha), with the description of *G. brayi* n. sp.*Syst. Parasitol.*, 68(1): 1 – 31. DOI: 10.1007/s11230-006-9082-2

Bray, R.A. (2005a): Family Acanthocolpidae Lühe (1906): In: Jones, A., Bray, R.A., Gibson, D.I. (Eds) *Keys to the Trematoda*. Volume 2. London, UK: CABI Publishing and The Natural History Museum, pp. 603 – 619

Bray, R.A. (2005b): Family Fellodistomidae Nicoll, 1909. In: Bray, R.A., Gibson, D.I., Jones, A. (Eds) *Keys to the Trematoda*. Volume 3. London, UK: CABI Publishing and The Natural History Museum, pp. 261 – 293

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W. (1997): Parasitology meets ecologyon its own terms: Margolis *et al*. revisited. *J. Parasitol.,* 83: 575 – 583. DOI: 10.2307/3284227

Cabral, H.N., (2000): Comparative feeding ecology of sympatric *Solea solea* and *S. senegalensis*, within the nursery areas of the Tagus estuary, Portugal. *J. Fish Biol.,* 57: 1550 – 1562. DOI: 10.1006/jfbi.2000.1408

Campbell, R.A, Beveridge, I. (1994): Order Trypanorhyncha Diesing, 1863. In: Khalil L.F., Jones A., Bray R.A. (Eds) *Keys to the cestode parasites of vertebrates*. CAB International, Wallingford, pp 51 – 148

Combes, C. (2001): *Parasitism: The Ecology and Evolution of Intimate Interactions.* University of Chicago Press, Chicago.

Demirkale, I., Özak, A. A., Yanar, A., Boxshall, G. A. (2014): *Caligus solea* n. sp.(Copepoda: Caligidae) parasitic on the common sole *Solea solea* (Linnaeus) from the north-eastern Mediterranean off the Turkish coast. *Syst. Parasitol.*, 89(1): 23 – 32. DOI: 10.1007/s11230-014-9505-4

Diaz de Astarloa, J. M. (2002): A review of the flatfish fisheries of the south Atlantic Ocean. *Rev Biol Mar Oceanogr*, 37(2): 113 – 125

Fanelli, E. (2008): *Trophic Relationships in Demersal Communities of Western Mediterraneo Occidentale: Case Studies From Coastal and Deep-sea Ecosystems*. Ph D Thesis, Italy: Universita´ degli Studi di Viterbo ‘‘La Tuscia’’, ICM-CSIC, 327 p.

Fanelli, E., Badalamenti, F., D’anna, G., Pipitone, C. (2009): Diet and Trophic Level of Scaldfish *Arnoglossus Laterna* in The Southern Tyrrhenian Sea (Western Mediterranean): Contrasting Trawled Versus Untrawled Areas. *J. Mar. Biolog. Assoc. U.K.*, 89(4): 817 – 828. DOI: 10.1017/S0025315409000137

Gibson, D.I. (2002): Family Hemiuridae Looss, 1899. In: Gibson, D.I., Jones, A., Bray, R.A. (Eds) Keys to the Trematoda. Volume 1. London, UK: CABI Publishing and The Natural History Museum, pp. 305 – 340

Gibson, R.N. (2005): *Flatfishes. Biology and Exploitation*. Oxford, UK, Blacwell Publishing, 391 pp.

Güneydağ, S., Özkan, H., Özer, A. (2017): *Scolex pleuronectis* (Cestoda) Infections in Several Bony Fish Species Collected from Sinop Coasts of the Black Sea. *Sinop Uni J Nat Sci.,* 2(1): 150 – 158

Kayış, Ş., Ceylan, Y. (2011): First report of *Nerocila orbigyni* (Crustacea, Isopoda, Cymothoidae) on *Solea solea* (Teleostei, Soleidae) from Turkish Sea. *Turk J Fish Aquat Sci,* 11: 167 – 169. DOI: 10.4194/trjfas.2011.0123

Keser, R. Bray, R.A., Oguz, M.C., Çelen, S., Erdoğan, S., Doğuturk, S., Aklanoğlu, G., Marti, B. (2007): Helminth parasites of digestive tract of some teleost fish caught in the Dardanelles at Çanakkale, Turkey. *Helminthologia*, 44 (4): 217 – 221. DOI: 10.2478/s11687-007-0035-3

Keskin, E., Koyuncu, C. E., Genc, E. (2015): Molecular identification of *Hysterothylacium aduncum* specimens isolated from commercially important fish species of Eastern Mediterranean Sea using mtDNA cox1 and ITS rDNA gene sequences. *Parasitol. Int.*, 64(2): 222 – 228. DOI: 10.1016/j.parint.2014.12.008

Lèbre, C., Petter, A. J. (1984): *Cucullanus campanae* n. sp. (Cucullanidae, Nematoda), parasite de la Sole *Solea vulgaris vulgaris* (Pleuronectiformes). *Bull. Mus. natn. Hist. nat.,* 4(6): 999 – 1005.

Køie, M. (1993): Aspects of the life cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda,Ascaridoidea, Anisakidae). *Can. J. Zool.*, 71: 1289 – 1296

Køie, M. (2001a): The life cycle of *Dichelyne* (*Cucullanellus*) *minutus* (Nematoda: Cucullanidae). *Folia Parasitol.*, (48); 304 – 310. DOI: 10.14411/fp.2001.048

Køie, M. (2001b): The life-cycle of *Capillaria gracilis* (Capillaridae), a nematode parasite of gadoid fish. *Sarsia*, 86: 383 – 387. DOI: 10.1080/00364827.2001.10425525

Kvach, Y., Oğuz, M. C. (2010): *Solearhynchus kostylewi* (Meyer, 1932) comb. nov.(Acanthocephala: Echinorhynchidae), a rare parasite of *Solea solea* (Pisces: Soleidae) in the Gemlik Bay, Sea of Marmara. *Parasite*, 17(1): 47 – 51. DOI: 10.1051/parasite/2010171047

MacKenzie, K., Abaunza, P. (2005): *Parasites as biological tags. In Stock Identification Methods. Applications in Fisheries Science* (eds S.X. Cadrin, K.D. Friedland & J.R. Waldman). San Diego, USA., Elsevier Academic Press, pp. 211 – 226

Marques, J.F., Santos, M.J., Teixeira, C.M., Batista, M.I., Cabral, H.N. (2011). Host-parasite relationships in flatfish (Pleuronectiformes) – the relative importance of host biology, ecology and phylogeny. *Parasitol.*, 138(1): 107 – 121. DOI: 10.1017/S0031182010001009

Miller, T.I., Cribb, T.H. (2008): Family Cryptogonimidae Ward, 1917. In: Bray, R.A., Gibson, D.I., Jones, A. (Eds) *Keys to the Trematoda*. Volume 3. London, UK: CABI Publishing and The Natural History Museum, pp. 51 – 112

Moravec, F. (1994): *Parasitic Nematodes of Freshwater Fishes of Europe*. Praha and Dordrecht, Boston, London, Academia and Kluwer Acad. Publishers, pp. 473

Muus, B. J., Dahlström, P. (1991): *Marine Fishes of the Baltic and North Seas, and Atlantics – Biology, Fishery and Economy*. *BLV-Key-Book*, 6 ed. Munich: Verllagsgesselschaft mbH.

Nielsen, J. (1973): Bothidae. In: Hureau, J.C., Monod, T. (Eds) Check List of Fishes of The North-Eastern Atlantic and of The Mediterranean. Paris, UNESCO, pp. 620 – 622

Özak, A. A., Demirkale, İ., Boxshall, G. A., Etyemez, M. (2013): Parasitic copepods of the common sole, *Solea solea* (L.), from the Eastern Mediterranean coast of Turkey. *Syst. Parasitol.,* 86(2): 173 – 185. DOI: 10.1007/s11230-013-9441-8

Özer, A., Öztürk, T., Kornyushin, V.V., Kornyychuk, Y., Yurakhno, V. (2014): *Grillotia erinaceus* (van Beneden, 1858) (Cestoda:Trypanorhyncha) from whiting in the Black Sea, with observations on seasonality and host-parasite interrelationship. *Acta Parasitol.*, 59(3): 420 – 425. DOI: 10.2478/s11686-014-0261-z

Özer, A., Özkan, H., Güneydağ, S., Yurokhno, V. (2015): First Report of Several Myxosporean (Myxozoa) and Monogenean Parasites from Fish Species off Sinop Coasts of the Black Sea. *Turk J Fish Aquat Sci,* 15(3): 737 – 744. DOI: 10.4194/1303-2712-v15_3_18

Pearson, J. (2008): Family Heterophyidae Leiper, 1909. In: Bray, R.A., Gibson, D.I., Jones, A. (Eds) *Keys to the Trematoda*. Volume 3. London, UK: CABI Publishing and The Natural History Museum, pp. 113 – 141

Pronkina, N.K, Dmitrieva, E.V., Polyakova, T.A., Popyuk, M.P. (2017): The Lifecycle of *Dichelyne minutus* (Rudolphi, 1819) (Nematoda: Cucullanidae) in the Estuarine Biocenosis of the Black Sea. *Russ J Mar Biol,* 43(2): 148 – 155. DOI: 10.1134/S1063074017020079

Zander, C.D. (2003): Four-year monitoring of parasite communities in gobiid fishes of the south-western Baltic: I. Guild and component community. *Parasitol. Res.*, 90: 502 – 511. DOI: 10.1007/s00436-003-0887-5

Zander C.D. (2004): Four-year monitoring of parasite communities in gobiid fishes of the south-western Baltic. II. Infracommunity. *Parasitol. Res.*, 93: 17 – 29. DOI: 10.1007/s00436-004-1087-7

DOI 10.2478/helm-2019-0005

HELMINTHOLOGIA, 56, 2: 141 – 150, 2019

Prevalence and intensity of *Cornudiscoides agarwali* (Monogenoidea) on the gills of Day's mystus (*Mystus bleekeri*) in relation to some ecological and biological factors from Arunachal Pradesh, India

A. TRIPATHI[1]*, L. WANGCHU[2], A. K. TRIVEDI[3]

[1]*Department of Zoology, University of Lucknow, Lucknow-226 007, E-mail: *amit.tripathi@rgu.ac.in*; [2]Department of Zoology, Rajiv Gandhi University, Arunachal Pradesh-791 112, India, E-mail: *lekiwangchuk7@gmail.com*; [3]Department of Zoology, Mizoram University, Mizoram- 796 004, India, E-mail: *amittrivedi9@gmail.com*

Article info

Received March 30, 2018
Accepted January 28, 2019

Summary

This work investigated the relationship of host size, seasons, and water quality parameters with the prevalence and intensity of *Cornudiscoides agarwali* on *Mystus bleekeri* collected from the Dikrong River in Arunachal Pradesh, India from February 2016 to January 2017. A total of 2760 specimens of *C. agarwali* were recovered from 114 individuals of *M. bleekeri*. The levels of mean intensity, but not the prevalence, of infection of *C. agarwali* were positively correlated with fish host size, peaking in the largest size class (45.20 ± 5.69 parasites/fish). The prevalence values had a statistically significant seasonal trend, reaching highest (100 %) during the pre-monsoon season, followed by 91.8% during the post-monsoon period and 87.5 % during the monsoon season. The levels of mean intensity of infection were also dependent on the seasons, reaching significantly higher levels during the pre-monsoon season (42.75 ± 4.18 parasites/fish). All water quality parameters measured were within the safety value recommended for freshwater aquaculture. *Cornudiscoides agarwali* maintained its prevalence above 87.5 % throughout the annual cycle, which means it was able to reproduce year-round in a non-polluted river. This could be an indication of monogenoidean community and population dynamics thriving best under optimum water quality parameters. Also, this article draws the attention of parasitologists and ichthyologists to a taxonomic problem of the misidentification of *Mystus* spp., and therefore, possibly of their parasitic monogenoids.

Keywords: Monogenoidea; *Mystus bleekeri*; *Cornudiscoides agarwali*; season; host size; 28S rRNA

Introduction

Fish diseases, especially those caused by parasitic monogenoids (Platyhelminthes), are one of the most important factors that threaten fish culture and fish farming globally (Thoney & Hargis, 1991). Monogenoids are very diverse ectoparasites, with more than 10,000 extant species distributed across the globe (as cited in Tripathi, 2014) and about 300 from India (Pandey & Agrawal, 2008). Arunachal Pradesh, located in the extreme northeast corner of India, has been identified as a major hotspot for freshwater fish biodiversity in the world (Kottelat & Whitten, 1996), boasting no fewer than 213 recorded species of fish (Bagra *et al*., 2009). Although only eight monogenoid species have been described/recorded from Arunachal Pradesh to date (Wangchu *et al*., 2017), more than 128 species have been identified from 55 species of sampled fish, giving an average infection of 2.32 monogenoids per infected host fish species (Tripathi: unpublished data).

Day's mystus (*Mystus bleekeri*) (Siluriformes: Bagridae) is a commercially important indigenous freshwater potamodromous fish species, which commonly inhabits the rivers and lakes of several

* – corresponding author

Asian countries, including Bangladesh, Bhutan, India, Indonesia, Myanmar, Nepal, and Pakistan (Froese & Pauly, 2018). In India, this species is known from the Brahmaputra-Ganges system and Indus and Mahanadi River drainages, where it is targeted as a food fish besides being exported as an ornamental fish (Ng, 2010). Despite the known importance of *M. bleekeri*, there is insufficient information regarding the distribution, biology, and potential threats for this species, including parasites (see Froese & Pauly, 2018). Only three species of helminth parasites (all monogenoids) have been recorded from *M. bleekeri*: *Cornudiscoides bleekerai* Agrawal and Vishwakarma, 1996, *C. gussevi* Agrawal and Vishwakarma, 1996, and *C. susanae* Agrawal and Vishwakarma, 1996 (Lim *et al*., 2001). Moreover, there is a complete lack of epidemiological data on the prevalence and intensity of parasites of *M. bleekeri* in relation to ecological and biological factors, which may hamper control interventions. Thus, we aimed to fill these knowledge gaps by determining the prevalence and intensity of monogenoids from *M. bleekeri* in relation to host size, season, and water quality parameters of the Dikrong River in Arunachal Pradesh, India.

Materials and Methods

Study area and host sampling

This study was conducted from February 2016 to January 2017 in the Dikrong River, a perennial tributary of the Brahmaputra River in western Arunachal Pradesh, India (27°6'N; 93°37'E) (Fig. 1). This river is approximately 145 km long (Huda, 2017) and has a catchment area of about 1,556 sq. km (Pandey *et al*., 2008). A total of 114 live specimens of *M. bleekeri* were collected using various local fishing nets and gears (see Chetry *et al*., 2012 for details) at monthly intervals from a single site to control for climatological conditions. Identification of fish specimens as *M. bleekeri* followed the taxonomic keys recommended by FishBase (Froese and Pauly 2017) and Jayaram (1999). They were immediately transferred to the research laboratory at Rajiv Gandhi University, Itanagar, where they were maintained in glass aquaria conditions, unless sacrificed, usually within 24 hours of collection, by anaesthetizing and then *pithing*. All fish specimens were measured (fork length, in cm) and classed into three length classes: 4.1 – 6 cm, 6.1 – 8 cm, and 8.1 – 10 cm to determine whether a correlation existed between fish length and infection level.

Water sampling and laboratory analysis

Water samples were collected monthly at the same time and place as host fish and were analysed according to American Public Health Association standards (APHA, 1998) for dissolved oxygen, Free CO2, Alkalinity, and pH. The surface water temperature was measured using an infrared thermometer (Mextex DT-8811). A one-year cycle was divided into three seasons (modified from Deka *et al*., 2015) – namely, pre-monsoon (February to May), monsoon (June to September), and post-monsoon (October to January).

Parasite sampling

Gills were dissected out and placed in petri dishes and examined for the presence and quantification of monogenoids under a dissecting microscope (Leica© EZ 4HD). Worms were picked off the gills using fine needles and then identified by a light microscope equipped with phase contrast optics using identification keys provided by Gussev (1976). After identification, some of the worms were fixed in 4 % formalin (for morphological studies) and others in absolute ethanol (for molecular studies). Temporary mounts were prepared by clearing and mounting the worms in glycerin for studying their sclerotized body parts; permanent mounts were made by staining and mounting the worms in Canada balsam according to the procedures recommended by Kritsky *et al*. (1986) for studying the soft body parts. The mounted parasites were photographed with a digital camera (Leica DFC450 C) attached to a Leica DM3000 light microscope. Voucher specimens were deposited in the British Natural History Museum, UK (NHMUK 2018.12.18.1).

DNA extraction, PCR, and sequencing

For molecular identification of parasite species, genomic DNA was extracted (from 52 adult worms fixed in 100 % ethanol) and 28S rDNA sequence was amplified following recent useful protocols (Tripathi *et al*. 2014; Choudhary *et al*. 2017). Sequencing was carried out by the commercial sequencing company (Xcelris Labs Limited, India) using the primers applied for PCR. The generated sequence (GenBank accession number MG832102.1) was subjected to BLAST (Basic Local Alignment Search Tool) analysis for homology search.

Table 1. Prevalence and mean intensity of infection of *Cornudiscoides agarwali* from *Mystus bleekeri* in relation to host size.

Size class	I	II	III
Length (cm)	4.1-6	6.1-8	8.1-10
Fish screened	29	54	31
Fish find infected	27	50	29
No. of parasites recovered	481	968	1311
Prevalence (%)	93.10%	92.59%	93.54%
Mean intensity (SE)	17.81 (3.02)	19.36 (2.42)	45.20 (5.69)

Table 2. Infection indices (in relation to three seasons and water quality parameters) of *Cornudiscoides agarwali* on *Mystus beleekri* from the Dikrong River, Arunachal Pradesh during 2016-2017 [DO= dissolved oxygen, Alk=Alkalinity, SWT=surface water temperature, FS=fish sampled, FI=fish infected, PR=parasites recovered, P=prevalence, MI=mean intensity].

Seasons	Water quality parameters					Host size classes			
Pre Monsoon	DO	Alk	pH	SWT		CL-I	CL-II	CL-III	Total
Feb	7.5	47.13	7.1	22.7	FS	8	18	7	33
March	6.2	52	7.2	25.2	FI	8	18	7	33
April	6.2	27.3	6.4	21	PR	254	690	467	1411
May	6.5	35.33	6.8	22	P (%)	100	100	100	100
Mean	6.6	40.44	6.87	22.72	MI	31.75	38.33	77.83	42.75
Monsoon									
June	6.8	27.6	7.2	24	FS	10	14	8	32
July	7.7	31.66	7	28	FI	8	12	8	28
August	8.2	32	7.1	28.8	PR	92	76	292	460
Sept	7.8	43.33	7.4	25.7	P (%)	80	85.71	100	87.50
Mean	7.6	33.68	7.17	26.62	MI	11.5	6.3	36.5	16.42
Post Monsoon									
Oct	6.9	33.66	6.6	21.3	FS	11	22	16	49
Nov	6	31.33	7.4	15.8	FI	11	20	14	45
Dec	6.2	22.33	7.9	16.8	PR	135	202	552	889
Jan	6.2	30.5	7.2	18	P (%)	100	90.90	87.5	91.8
Mean	6.2	29.45	7.27	17.85	MI	12.27	10.1	39.42	19.75

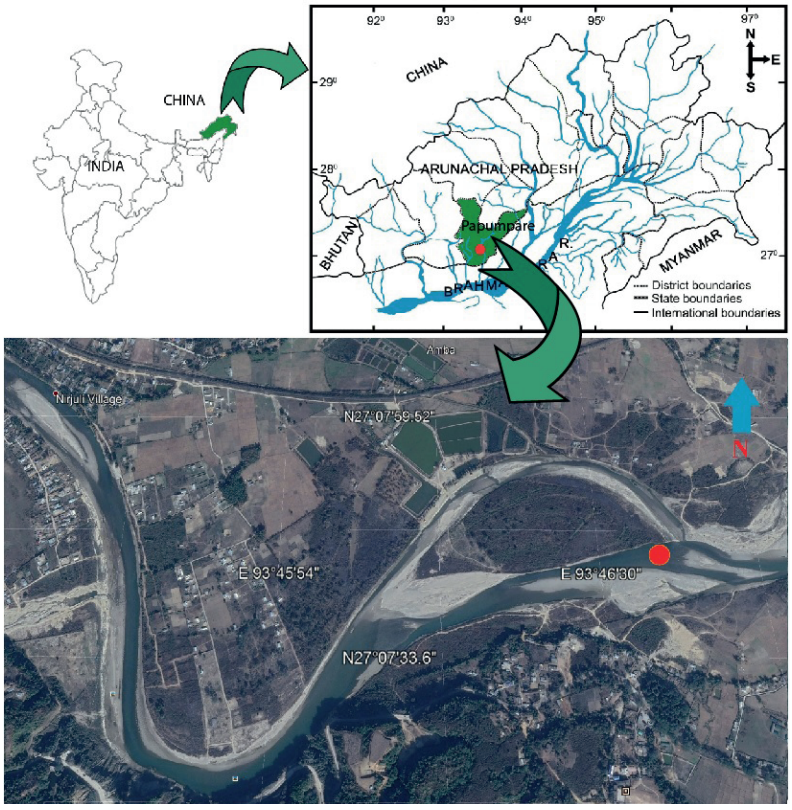

Fig. 1. The map of collection site of *Mystus bleekeri* in Arunachal Pradesh, India (satellite image from Google Earth™).

Fig. 2. Photomicrograph of an individual of *Mystus bleekeri* (not drawn to scale). Photo by Leki Wangchu.

Statistical analysis

The infection variables studied were prevalence (percentage of infected hosts in a sample) and mean intensity (mean number of parasites per infected host in a sample), which were calculated according to Bush *et al*. (1997). The standard deviation was calculated using Microsoft Excel (Office 2010). Fisher exact test was used for testing differences in prevalence values between the seasons. Kruskal-Wallis (K-W) ANOVA test was used for comparing the variations in the mean intensity of parasites for the seasons and host fish size. Pearson's correlation test was used to measure the correlation between mean intensity of infection and host fish size. All statistical calculations and graphs were made in the GraphPad Prism software (version 6). Confidence limits (P –values) were set at 95 %.

Ethical Approval and/or Informed Consent

The research related to animal use has been complied with all the relevant institutional policies for the care and use of animals.

Results

114 individuals of *M. bleekeri* (Fig. 2) were collected from February 2016 to January 2017 in the Dikrong River in western Arunachal Pradesh, northeast India. Upon the parasitological analysis of collected samples, two monogenoid species were found and identified using morphological characters: *Cornudiscoides agarwali*

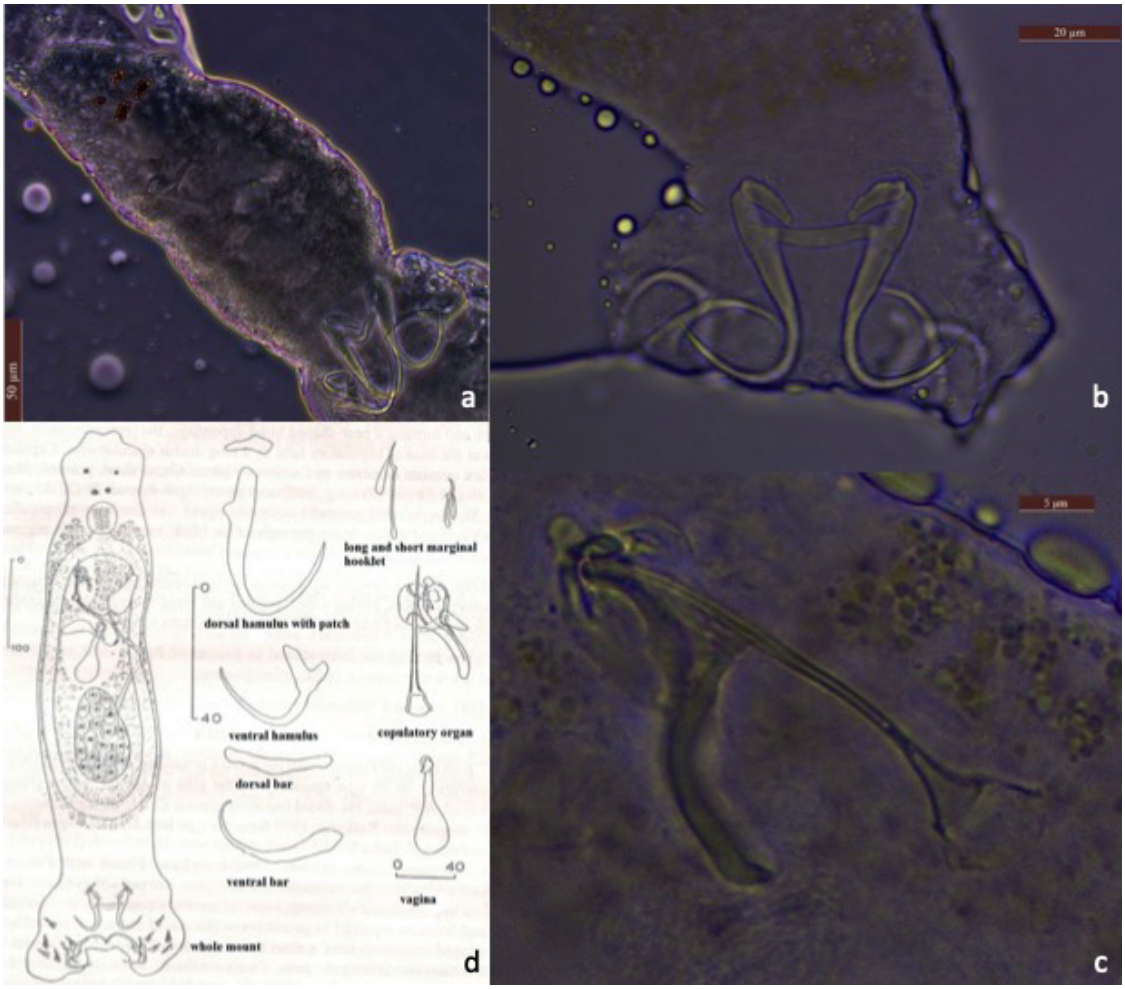

Fig. 3. Montage of *Cornudiscoides agarwali* recovered from *Mystus bleekeri*. (a) An individual worm, dorsal view. (b) Haptor armed with sclerotized structures: anchors, bars and hooks. (c) Copulatory complex. (d) Original figures of *Cornudiscoides agarwali* by Agrawal and Vishwakarma (1996). Scale bar: 100 µm for 3a, 20 µm for 3b and 5 µm for 3c. Photo (a, b, and c) by Amit Tripathi.

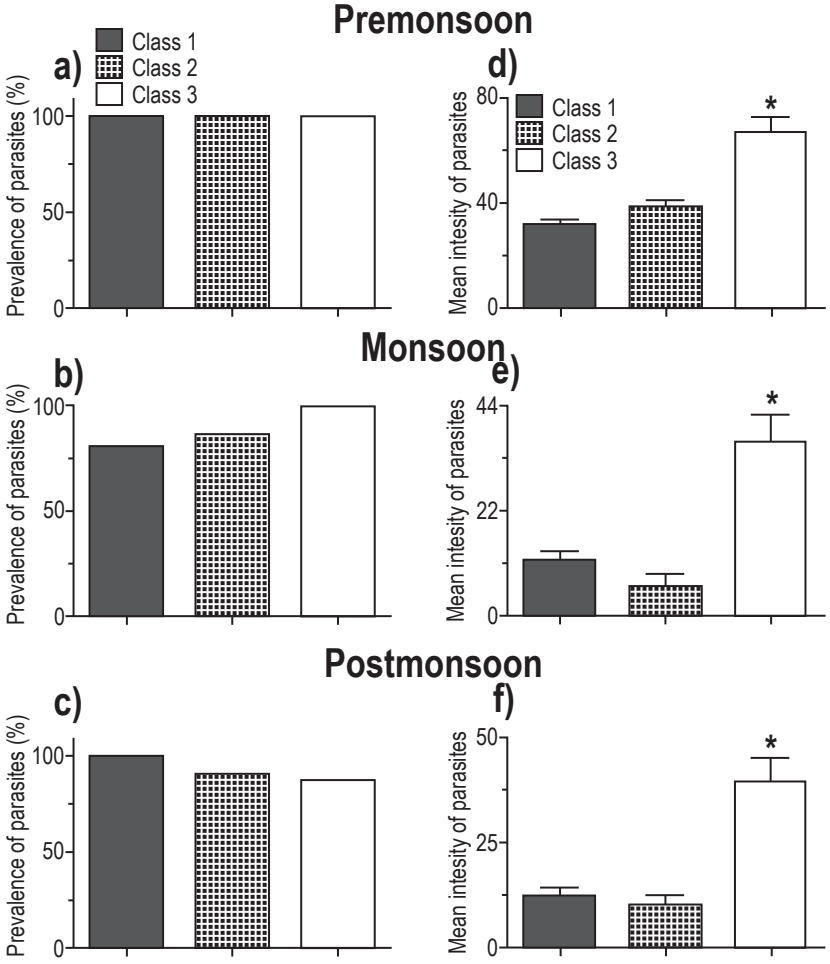

Figure 4. Left hand panel shows prevalence of parasites (a, b, c) and right hand panel shows mean intensity of parasites (d, e, f) during different seasons. Top panel shows prevalence and mean intensity during pre-monsoon (a, d), middle panel shows prevalence and mean intensity during monsoon (b, e), and bottom panel shows the prevalence and mean intensity during the post-monsoon season (c, f). * indicates significant difference (P < 0.05).

Agrawal and Vishwakarma, 1996 (Fig. 3) and *Cornudiscoides* n. sp. Of these two species, only *C. agarwali* was found infesting throughout the investigation period covering all host sizes and seasons, and hence, it was the subject of the current investigation. Of the 114 specimens of *M. bleekeri* examined, 106 were found to be infested with 2760 individuals of *C. agarwali* with overall prevalence and mean intensity values of 92.98 % and 26.03 ± 4.43, respectively. The results of molecular analysis showed that the 28S rRNA gene sequence for *C. agarwali* from the present study (accession MG832102.1) was most closely related to *C. agarwali* from northern India (query cover 95 %, E value 0, max identity 92 %, accession KU208071.1, and query cover 25 %, E value 5e-84, max identity 98 %, accession KU208072.1).

Size classes of the host fish and the distribution of population of C. agarwali

The prevalence levels of *C. agarwali* were almost similar, at 93.10 %, 92.59 %, and 93.54 % in the three host fish size classes of 4.1 – 6 cm, 6.1 – 8 cm, and 8.1 – 10 cm, respectively (Table 1). The mean intensity was, however, highest in the fish of class III (8.1 – 10 cm) (45.20 ± 5.69 parasites/fish), followed by class II (6.1 – 8 cm) (19.36 ± 2.42 parasites/fish) and class I (4.1 – 6 cm) (17.81 ± 3.02 parasites/fish) (Table 1). The Kruskal-Wallis test revealed a significant variance in the mean intensity of infection between class I (4.1 – 6 cm) and class III (8.1 – 10 cm) ($P<0.0001$) and between class II (6.1 – 8 cm) and class III (8.1 – 10 cm) ($P<0.0001$); there was no difference between class I (4.1 – 6 cm) and class II (6.1 – 8 cm). Bonferroni post hoc test indicated that the mean intensity was significantly higher ($P<0.05$) in class III in comparison to class I and II. Pearson's correlation also suggested a positive correlation ($r=0.12$, $P=0.0002$; $n=114$) between mean intensity of parasite infection and host size.

Seasonal distribution of population of C. agarwali

The infestation of *M. bleekeri* with *C. agarwali* was found throughout the year though, with a definite seasonal effect. The prevalence

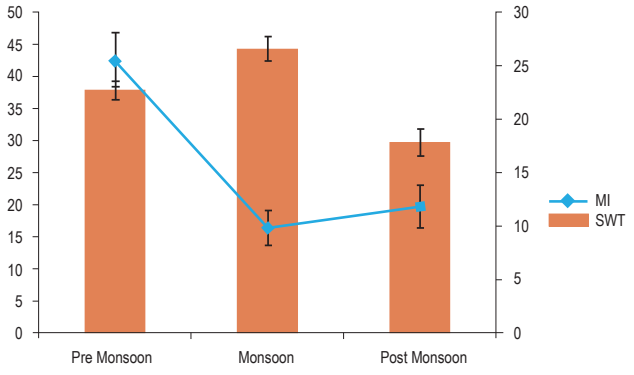

Fig. 5. Seasonal changes in the mean intensity of *Cornudiscoides agarwali* on the gills of *Mystus bleekeri* in relation to surface water temperature.

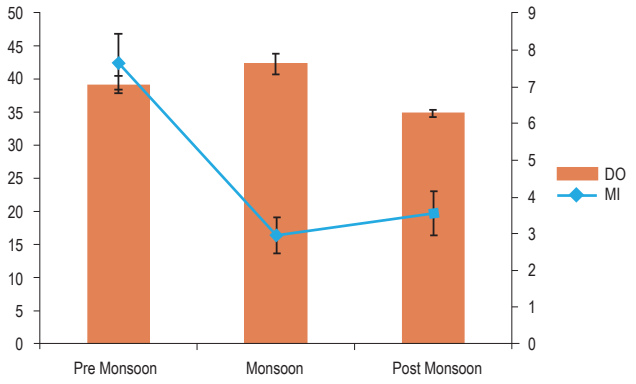

Fig. 6. Seasonal changes in the mean intensity of *Cornudiscoides agarwali* on the gills of *Mystus bleekeri* in relation to dissolved oxygen.

was highest (100 %) during the pre-monsoon season, followed by 91.8 % during the post-monsoon period and 87.5 % during the monsoon season (Table 2). A Fisher's exact test revealed that the differences in the prevalence of the monogenoids among the different seasons were significant (P<0.0001) (Fig. 4). Seasonal changes in the mean intensity also showed significant variations [$F_{2,104}$=14.2, P<0.0001 (one way analysis of variance]. For all three class sizes, the mean intensity was lowest (16.42 ± 2.90 parasites/fish) during the monsoon season, followed by an increase (19.75 ± 3.32 parasites/fish) in the post-monsoon season, and the highest (42.75 ± 4.18 parasites/fish) during the pre-monsoon season (Table 2).

Water quality parameters and the seasonal distribution of population of C. agarwali

Surface-water temperature

Surface-water temperature (°C) in the sampling area had a mean (± SE) of 22.4 ± 1.22 °C. The highest mean temperature was recorded in August (28.8 ± 0.38 °C) and the lowest in November (15.8 ± 0.07 °C). The highest mean intensity of *C. agarwali* was recorded during the pre-monsoon period (42.75 parasites/fish) when the surface-water temperature was 22.72 °C. Then, it rapidly declined during the monsoon period (16.42 parasites/fish) when the surface-water temperature increased to 26.62 °C. It increased slightly during the post-monsoon period (19.75 parasites/fish) when the surface-water temperature decreased to 17.85 °C (Fig. 5).

Dissolved oxygen

Dissolved oxygen (DO) (mg/L) in the sampling area showed a mean (± SE) of 6.83 ± 0.22 mg/L. The highest DO mean value was recorded in August (8.2 ± 0.11) and lowest in October (6 ± 0.11). The mean intensity of infection was highest during the pre-monsoon period (42.75 parasites/fish) when the mean DO was 6.6 mg/L. It was followed by a rapid decline during the monsoon period (16.42 parasites/fish) when the mean DO increased to 7.62 mg/L. Then, it increased slightly during the post-monsoon period (19.75 parasites/fish) when the mean DO decreased to 6.27 mg/L (Fig. 6).

Water pH

The pH in the sampling area showed a mean (± SE) of 7.10 ± 0.11. The highest pH mean value was recorded in December (7.9 ± 0.05), and lowest in April (6.4 ± 0.12). The mean intensity of infection was highest during the pre-monsoon period (42.75 para_ sites/fish) when the mean pH as 6.87. It was followed by a rapid

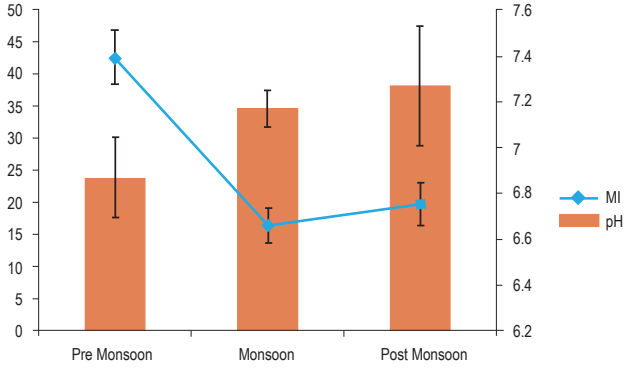

Fig. 7. Seasonal changes in the mean intensity of *Cornudiscoides agarwali* on the gills of *Mystus bleekeri* in relation to pH.

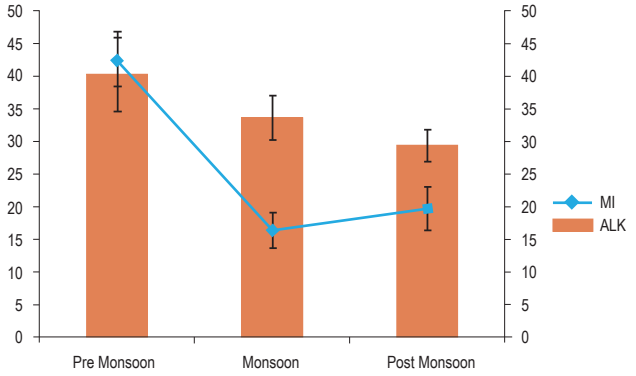

Fig. 8. Seasonal changes in the mean intensity of *Cornudiscoides agarwali* on the gills of *Mystus bleekeri* in relation to alkalinity.

decline during the monsoon period (16.42 parasites/fish) when the mean pH increased to 7.17. The mean intensity of infection then increased slightly (19.75 parasites/fish) during the post-monsoon period when the mean water pH also slightly increased to 7.27 (Fig. 7).

Water alkalinity

Alkalinity (mg/L) in the area showed a mean (± SE) of 34.51 ± 2.51 mg/L. The highest mean alkalinity was recorded in February (52 ± 1.99) and the lowest in December (22.33 ± 0.95). The mean intensity of infection was highest during the pre-monsoon period (42.75 parasites/fish) when the mean alkalinity was around 40.44 mg/L. This was followed by a rapid decline during the monsoon period (16.42 parasites/fish) when the mean alkalinity decreased to 33.64 mg/L. However, the mean intensity of infection increased slightly during the post-monsoon period (19.75 parasites/fish) when the mean alkalinity further decreased to 29.45 mg/L (Fig. 8).

Discussion

Given the high host specificity of monogenoids (Desdevises *et al*., 2002) it was highly improbable, though not impossible, that *C. agarwali* originally described from *M. vittatus* in northern India would infect specimens of *M. bleekeri* in Arunachal Pradesh in extreme northeast India. While the near identical morphometry of *C. agarwali* from Lucknow (1996) and Itanagar (this study) (Fig. 3) strongly suggested that they are one and the same species, we were also sure that our identification of *M. bleekeri* was correct. We then attempted, therefore, to identify parasite species via PCR and DNA sequencing. The partial nucleotide sequence (892 bps) of the 28S rRNA gene of *C. agarwali* from the present study (accession MG832102.1) was characterized and compared with previously available *Cornudiscoides* species. The BLASTn search showed two entries that returned the same species name for 28S rRNA gene: accession KU208071.1 (query cover 95 %, E value 0, max identity 92 %) and accession KU208072.1 (query cover 25 %, E value 5e-84, max identity 98 %). On balance, these are inconclusive BLASTn results as to whether the worms in question are of same species-level taxon.

A thorough search of the literature on the taxonomy and distribution of *Mystus* spp. suggests that this problem could be due to the misidentification of the Indian species of *Mystus* and, thus, their monogenoids. For example, despite the fact that *M. tengara* is a north Indian species (Jayaram & Sanyal 2003), Kulkarni (1969) described *Cornudiscoides heterotylus*, the type species of the genus, from *M. tengara* in Hyderabad (south India). Similarly, Agrawal and Vishwakarma (1996) described *C. agarwali* from *M. vittatus* in Lucknow (north India), although *M. vittatus* is a south Indian species. Likewise, *Mystus keletius* is confined to southern India, and possibly Sri Lanka, only (Dahanukar, 2011), yet *M. keletius* has been found in Bengal and north India (Johal & Tandon 1979; Jayaram & Sanyal 2003 and references therein). These taxonomic errors can be appreciated in the background of the fact that the identification of many species within the genus *Mystus*, which has traditionally been based on morphological features, is very difficult and confusing (Jayaram & Sanyal 2003; Singh *et al*.; 2013; Plamoottil, 2017) and their phylogeny is also unclear (Singh *et al*., 2013). For example, Jayaram and Sanyal (2003) noted that '*M. vittatus* and *M. tengara* are closely related and their exact identity has been in confusion'. Darshan *et al*. (2010) also pointed out that 'several records of *M. vittatus* from northeast India and Gangetic basin (in northern India) were misidentifications of *M. tengara*'. In fact, *M. vittatus* may have been confused with many other *Mystus* species (Froese & Pauly 2018; Hossain, 2014). Similarly, the identity of *Mystus nigriceps* Valenciennes has also been the subject of much confusion among ichthyologists (Ng, 2002).

In view of the high host specificity of monogenoids combined with the widespread confusion over the identity of *Mystus* spp., and the identical morphometry of *C. agarwali* from Lucknow (1996) and Itanagar (this study), we believe that Agrawal and Vishwakarma (1996) might have confused *M. bleekeri* with (and misidentified it as) *M. vittatus*. This explains the occurrence of *C. agarwali* in *M. bleekeri* in Arunachal Pradesh. However, examination of additional genes, probably more rapidly evolving markers such as *Cox1*, are necessary to confirm the validity of recognizing *C. agarwali* from northeast India and northern India. This is beyond the scope of this paper.

With respect to host size, the prevalence levels of *C. agarwali* were 93.10 % in class I (4.1 – 6), 92.59 % in class II (6.1 – 8), and 93.54 % in class III (8.1 – 10). Clearly, prevalence values were not dependent on host size. The mean intensity levels, however, generally increased with host size and peaked in the largest class size (8.1 – 10), indicating that the size of the host fish is important in determining the parasitic load. Statistically, this trend was more distinct between sizes classes I (4.1 – 6) and III (8.1 – 10) and between size classes II (6.1 – 8) and III (8.1 – 10). Our findings are similar to those reported by Tombi *et al*. (2014), who found the highest mean intensity of *Dactylogyrus amieti* and *Dogielius njinei* in larger fish (> 7.5 cm). These results could well be a reflection of any one or even a combination of the following factors: (i) that larger host species live longer, and thus, represent a more predictable resource for a parasite (Peters, 1983); (ii) that the larger-bodied hosts may be easier to colonize because of their larger roaming ranges, or (being older), their longer time to have accumulated parasites (Poulin, 1998), and (iii) that larger hosts offer more vacant niches for greater abundance of parasitic monogenoids (Guegan *et al* 1992; Sasal *et al*., 1999).

Cornudiscoides agarwali showed a seasonal variation in terms of prevalence, though the values remained above 87.5 % throughout the year, of course suggesting that the parasite reproduced around the year. On the other hand, the mean intensity values fluctuated for all the three class sizes from low (during the monsoon), to moderate (in the post-monsoon period), to high (in pre-monsoon period), generating a statistically significant seasonal cycle.

We investigated the physical (temperature) and chemical (DO, pH, and alkalinity) properties of the river water with respect to the mean intensity values of *C. agarwali* to understand the seasonal relationship between the infestation of monogenoids and the water quality parameters. With respect to water temperature, the results indicated an inverse relationship between mean intensity and water temperature during monsoon and post-monsoon seasons, and a direct relationship during the pre-monsoon season. Many studies have indicated that high water temperature often increases monogenoids' growth, reproduction, and egg hatching, facilitating the build-up of large populations (e.g., Hooglund & Thulin, 1989; Gannicott & Tinsley, 1998; Kim *et al*., 2001; Silan & Maillard, 1989). The direct relationship between mean intensity of infestation and the water temperature during the pre-monsoon season in this study supports this assumption, as did the findings of Anderson (1974) and Simkova *et al*. (2000). However, the inverse relationship found during the monsoon and post-monsoon seasons suggests that temperature alone may not be the only controlling factor in the population dynamics of *C. agarwali*. According to Marcogliese (2001) the low water levels and the subsequent low flow rates of water may promote the retention of free-swimming infective stages of complex life-cycle parasites. We suggest that the inverse relationship between *C. agarwali* and water temperature during the monsoon and post-monsoon seasons is actually a function of higher stream velocity, which may have swept away larvae (oncomiracidia) resulting in low recruitment. It is worth noting that an increase in velocity of Indian rivers due to heavy rainfall during the monsoon season is well documented (see Soni *et al*., 2014). This velocity increases even further in hill streams of Arunachal Pradesh (Mahanta *et al*., 2012) due to presence of steep slopes and the obstructions within the river beds.

With respect to the dissolved oxygen, the results indicated an inverse relationship between mean intensity and dissolved oxygen during the monsoon and post-monsoon seasons but a direct relation during the pre-monsoon season. It is important to note that, throughout the year of investigation, the concentrations of dissolved oxygen were found to be within the recommended range for freshwater fish in the tropics (desired values of DO>5 mg/L) (Boyd, 1982; Wetzel, 1983). Seemingly, the dissolved oxygen, when present in its optimum value, had little or no significant influence over the monogenoids' proliferation. The decrease in mean intensity of infestations during the monsoon and post-monsoon seasons was probably related to the higher stream velocity discussed above, and not to the dissolved oxygen.

With respect to the pH, the results indicated an inverse relationship for the pre-monsoon and monsoon seasons but a direct relation for the post-monsoon season. In this study, the seasonal range of pH (from 6.8 to 7.2) remained within a narrow range, making it difficult to understand or predict its effect on the seasonal dynamics of *C. agarwali*. Moreover, the pH values observed were close to recommended values (7 – 8) (Boyd, 1982; Wetzel, 1983) for tropical aquaculture. From these facts one would expect that *C. agarwali* survives and reproduces best in water with a near-neutral pH value.

With respect to water alkalinity, the results indicated a direct relationship between mean intensity and water alkalinity during the pre-monsoon and monsoon seasons but an inverse relationship during the post-monsoon season. The alkalinity is interdependent with other water quality parameters, especially pH. Since the observed pH values staryed within a narrow range across seasons, alkalinty did not seem to have any significant impact on the mean intensity of infection of *C. agarwali*. Moreover, the values of alkalinity were found to stay within the recommended range for freshwater catfish (>20 mg/l) throughout the annual cycle (Swann, 1997).

Considerable work has been done on the interaction between water quality parameters and parasitism, concluding with three major and often contradictory predictions. The first conclusion is that pollutants may increase parasitism by reducing the immunological response of hosts (McDowell *et al*., 1999), including fish (Jokinen *et al*., 1995; Siddall *et al*., 1996), thus rendering them more susceptible to some parasites. The second conclusion is that pollutants can decrease parasitism by killing the parasites directly or by reducing the host density by causing differential mortality in infected hosts but not in uninfected fish (reviewed by Sures 2004). The third conclusion is that eutrophication may increase parasitism by increasing the abundance of their hosts (Beer & German, 1993) including fish (Valtonen *et al*., 1997) through nutrients-associated productivity. The river water in the Indian Himalayan region, spread across 11 states including Arunachal Pradesh, is generally free from pollution, since the industrial and domestic pollutants are minimal in this region (Semwal *et al*., 2006; National Commission on Farmers 2004). This is justified by the fact that mean values – as well as extreme values – of water quality parameters tested in this study were all within the desired values for tropical aquaculture (see above). Therefore, the fact that *C. agarwali* was found reproducing throughout the year (since it maintained its prevalence above 87.5 % throughout the year) in a non-polluted Dikrong River site indicates that the monogenoidean community and population dynamics thrives best under optimum water quality parameters. To confirm this, however, laboratory-based experimental studies are necessary to determine the tolerance of *C. agarwali* to pollutants.

Conflict of Interest

Authors state no conflict of interest.

Acknowledgments

This study was financially supported by the Department of Science and Technology, Government of India (SR/SO/AS-56/2011) through a research grant to AT. We thank two anonymous reviewers for their many insightful comments and suggestions.

References

Agarwal, N., Vishwakarma, P. (1996): Six new species and redescription of two known species of *Cornudiscoides* Kulkarni, 1969 (Monogenea) from Lucknow, U.P. *Indian J. Helminth.* (*N.S.*), 13: 10 – 31

Anderson, R.M. (1974): An analysis of the influence of host morphometric features on the population dynamics of *Diplozoon paradoxum* (Nordmann, 1832). *J. Anim. Ecol.,* 43: 873 – 887. DOI: 10.2307/3541

American Public Health Association (1998): *Standard Method for the Examination of Water and Waste Water*. 20th Edition, Washington, USA, American Water Work Association and Water Environment Federation, 361 pp.

Bagra, K., Kadu, K., Sharma, K.N., Laskar, B.A., Sarkar, U.A., Das, D.N. (2009): Ichthyological survey and review of the checklist of fish fauna of Arunachal Pradesh, India. *Check List*, 5: 330 – 350. DOI: 10.15560/5.2.330

Beer, S.A., German, S.M. (1993): The ecological prerequisites for a worsening of the cercariasis situation in the cities of Russia (exemplified by the Moscow region). *Parazitologiia*, 27(6): 441 – 449.

Boyd, C.E. (1979): *Water Quality in Warm Water Fish Ponds*. Alabama, USA, Auburn University, 359 pp.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W. (1997): Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Int. J. Parasitol.*, 83: 575 – 583. DOI: 10.2307/3284227

Chetry, D.B., Narba, D., Tripathi, A. (2012): Traditional fishing methods in Arunachal Pradesh. In A. Tripathi (Ed.) *Proceedings of the 1st National Symposium on Fish Parasites Itanagar, Helminthological Society of India, March 19 – 20, 2012*. pp 41 – 44

Choudhary, K., Tripathi, A., Pandey, K.C., Agrawal, N. (2017): Morphological and molecular characterisation of three Indian *Neascus*-type metacercariae (Digenea: Diplostomidae). *Parasitol. Int*., 66: 652 – 659. DOI: 10.1016/j.parint.2017.06.007

Dahanukar, N. (2011): *Mystus keletius*. In: *IUCN Red List of Threatened Species 2011*: e.T172324A6867980. Retrieved March 18, 2018 from http://dx.doi.org/10.2305/IUCN.UK.2011–1.RLTS.T172324A6867980.en.

Darshan. A., Mahanta, P.C., Barat, A., Kumar, P. (2013): Redescription of the striped catfish *mystus tengara* (Hamilton, 1822) (Siluriformes: Bagridae), India. *J. Threat. Taxa*, 5(1): 3536 – 3541. DOI**:** 10.11609/JoTT.o2813.842

Deka, J.P., Tayeng, G., Singh, S., Hoque, R.R., Prakash, A., Kumar, M. (2015): Source and seasonal variation in the major ion chemistry of two eastern Himalayan high altitude lakes. *Arabian J. Geosci*., 8(12):10597 – 10610. DOI: 10.1007/s12517-015-1964-7

Desdevises, Y., Morand, S., Legendre, P. (2002): Evolution and determinants of host specificity in the genus Lamellodiscus (Monogenea). *Biol. J. Linn. Soc.*, 77: 431 – 443

Froese, R., Pauly, D. (2018): *Fish Base*. Retrieved March 2, 2018 from http://www.fishbase.org.

Gannicott, A.M., Tinsley, R.C. (1998): Larval survival characteristics and behaviour of the gill monogenean *Discocotyle sagittata*. *Parasitology*, 117: 491 – 498

Guegan, J.F., Lambert, A., Leveque, C., Combes, C., Euzet, L. (1992): Can host body size explain the parasite species richness in tropical freshwater fishes? *Oecol*., 90:197 – 204

Gussev, A.V. (1976): Freshwater Indian monogenoidea: Principles of systematics, analysis of the world faunas and their evolution. *Ind. J. Helminthol.*, 25 – 26: 1 – 241

Höglund, J., Thulin, J. (1989): Thermal effects on the seasonal dynamics of *Paradiplozoon homoion* (Bychowsky and Nagibina, 1959) parasitizing roach, *Rutilus rutilus* (L.). *J. Helminthol*., 63: 93 – 101. DOI: 10.1017/S0022149X0000883X

Hossain, M.Y. (2014): Threatened fishes of the world: (Bloch, 1794) (Siluriformes: Bagridae). *Croatian Journal of Fisheries,* 72: 183 – 185

Huda, E.A. (2017): Morphometric characteristics of Dikrong River catchment in the foot-hills of Arunachal Himalayas. *IOSR Journal of Humanities and Social Science,* 22(7): 51 – 60

Jayaram, K.C (1999): The Freshwater Fishes of the Indian Region Narendra Publishing House, pp551.

Jayaram, K.C., Sanyal, A. (2003): A taxonomic revision of the fishes of the genus *Mystus Scopoli*. (Family: Bagridae) records of the zoological survey of India. *Zoological Survey of India,* 207: 1 – 141

Johal M.S., Tandon, K.K. (1979): Monograph on the fishes of organised Punjab. Part I. *Punjab Fish Bulletin,* 3(2): 1 – 44

Jokinen, E.I., Aaltonen, T.M., Valtonen, E.T. (1995): Subchronic effects of pulp and paper mill effluents on the immunoglobulin synthesis of roach *Rutilus rutilus*. *Ecotoxicol. Environ. Saf.,* 32: 219–263. DOI: 10.1006/eesa.1995.1107

Kim, K.H., Ahn, K.J., Kim, C.S. (2001): Seasonal abundances of *Prosomicrocotyla gotoi* (Monogenea) and *Opecoelus sphaericus* (Digenea) from greenlings *Hexagrammos otakii* in a southern coastal area in Korea. *Aquaculture*, 192(2 – 4):147 – 153. DOI: 10.1016/ S0044-8486(00)00464-6

Kottelat, M., Whitten, T. (1996): Freshwater biodiversity in Asia with special reference to fish. In: *World Bank Tech*. Paper No. 343. The World Bank, Washington, DC., pp 17 – 22

Kritsky, D.C., Thatcher, V.E., Boeger, W.A. (1986): Neotropical Monogenea. Revision of *Urocleidoides* (Dactylogyridae, Ancyrocephalinae). *Proc. Helminthol. Soc. Wash.,* 53: 1 – 37

Lim, L.H.S., Timofeeva, T.A., Gibson, D.I. (2001): Dactylogyridean monogeneans of the siluriform fishes of the Old World. *Syst. Parasitol.*, 50: 159 – 197

Marcogliese, D.J. (2001) Implications of climate change for parasitism of animals in the aquatic environment. *Can J Zool.*, 2001, 79(8): 1331 – 1352. DOI: 10.1139/z01-067

Mahanta, P.C., Sarma, D. Dutta, R. (2012). Ecological Status and Fishery Potential of Deopani: a Coldwater Stream of Arunachal Pradesh. *The Clarion*, 1: 37 – 45

McDowell, J.E., Lancaster, B.A., Leavitt, D.F., Rantamaki, P., Ripley, B. (1999): The effects of lipophilic organic contaminants on reproductive physiology and disease processes in marine bivalve

molluscs. *Am. Soc. Limnol. Oceanogr.*, 44: 903 – 909
National Commission On Farmers (2004): Serving farmers and saving farming from crisis to confidence (second report), New Delhi. 523 pp. Retrieved from http://agricoop.nic.in/NCF/ NCF %20Report %20- %202
Ng, H.H. (2002): The identity of *Mystus nigriceps* (Valenciennes in Cuvier & Valenciennes, 1840), with the description of a new bagrid catfish (Teleostei: Siluriformes) from Southeast Asia. *Raffles B. Zool.,* 50: 161 – 168.
Ng, H.H. (2010): *Mystus bleekeri*. *2010 IUCN Red List of Threatened Species*: e.T166611A6247112.15. Retrieved February 2018 from http://dx.doi.org/10.2305/IUCN.UK. 20104.RLTS. T166611A6247112.en
Pandey, K.C., Agrawal, N. (2008): *An Encyclopedia of Indian Monogenoidea*. New Delhi, Vitasta Publishing Pvt. Ltd., 552 pp.
Pandey, A., Dabral, P.P., Chowdary, V.M., Yadav, N.K. (2008): Landslide hazard zonation using remote sensing and GIS: A case study of Dikrong River basin, Arunachal Pradesh, India. *Environ. Geol.*, 54: 1517 – 1529. DOI: 10.1007/s00254-007-0933-1
Peters, R. H. (1983): The ecological implications of body size. Cambridge University Press, Cambridge. DOI: 10.1017/CBO9780511608551
Plamoottil, M. (2017): Taxonomic notes on Mystus species of northern Kerala. *J. Aquacult. Res. Dev.*, 8: 495. DOI: 10.4172/2155-9546.1000495
Poulin, R. (1998): *Evolutionary Ecology of Parasites: From Individuals to Communities*. London, Chapman and Hall, 212 pp.
Sasal, P., Trouve, S., Muller-Graf, C., Morand, S. (1999): Specificity and host predictability: a comparative analysis among monogenean parasites of fish. *J. Anim. Ecol.*, 68, 437 – 444. DOI: 10.1046/j.1365-2656.1999.00313.x
Semwal, N., Akolkar, P. (2006): Water quality assessment of sacred Himalayan rivers of Uttaranchal. *Curr. Sci.*, 91: 486 – 496
Siddall, R., Koskivaara, M., Valtonen, E.T. (1996): Dactylogyrus (Monogenea) infections on the gills of roach (*Rutilus rutilus L.*) experimentally exposed to paper and pulp mill effluent. *Parasitology*, 114: 439 – 446. DOI: 10.1017/S003118209600875X
Silan, P., Maillard, C. (1989): Biologie comparée du développement et discrimination des Diplectanidae ectoparasites du Bar (Teleostei).[Comparative biology of development and discrimination of Diplectanidae, ectoparasites of sea bass (Teleostei)]. *Annls Sci. Nat.*, 10: 31 – 45 (In French)
Simkova, A., Desdevises, Y., Gelnar, M., Morand, S. (2000): Co-existence of nine gill ectoparasites (*Dactylogyrus*: Monogenea) parasitising the roach (*Rutilus rutilus* L.): history and present ecology. *Int. J. Parasitol.*, 30: 1077 – 1088. DOI: 10.1016/S0020-7519(00)00098-9
Singh, S.S., Chingakham, B.S., Gusheinzed, W. (2013): Karyotype Analysis of the New Catfish Mystus ngasep (Siluriformes: Bagridae) from Manipur, India. *Turk. J. Fish. Aquat. Sc.*, 13: 179 – 185. DOI: 10.4194/1303-2712-v13_1_21
Soni, V., Shekhar, S., Singh, D. (2014): Environmental flow for the Yamuna river in Delhi as an example of monsoon rivers in India. *Curr. Sci.*, 106: 558 – 564
Sures, B. (2004): Environmental Parasitology: Relevancy of parasites in monitoring environmental pollution. *Trends Parasitol.*, 20: 170 – 177. DOI:10.1016/j.pt.2004.01.014
Swann, L.D. (1997): *A Fish Farmer's Guide to Understanding Water Quality, Aquaculture Extension*. Illinois, USA, Purdue University, Indiana Sea Grant Program Fact Sheet AS-503.
Thompson, J. D., Higgins, D.G., Gibson, T.J. (1994): ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, 22 (22): 4673 – 4680. DOI: 10.1093/nar/22.22.4673
Thoney, D.A., Hargis, W.H.J. (1991): Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annu. Rev. Fish Dis.*, 1: 133 – 153. DOI: 10.1016/0959-8030(91)90027-H
Tripathi, A. (2014): The invasive potential of parasitic monogenoids (platyhelminthes) via the aquarium fish trade: An appraisal with special reference to India. *Rev. Aquacult.,* 6: 147 – 161. DOI: 10.1111/raq.12035
Valtonen, E.T., Holmes, J.C., Koskivaara, M. (1997): Eutrophication, pollution, and fragmentation: effects on parasite communities in roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in four lakes in central Finland. *Can. J. Fish Aquat. Sci.*, 54(3): 572 – 585, DOI: 10.1139/f96-306
Wangchu, L., Narba, D., Yassa, M., Tripathi, A. (2017): *Dactylogyrus barnae* sp. n. (Platyhelminthes: Monogenoidea) infecting gills of *Barilius barna* Hamilton, 1822 (Pisces: Cyprinidae) from a global biodiversity hotspot - Arunachal Pradesh (India). *Vet. World*, 10(5), 505 – 509. DOI:10.14202/vetworld.2017.505-509
Wetzel, R.G. (1983): *Limnology*. 2nd Edition, Philadelphia, USA, Saunders College Publishing

sciendo

DOI 10.2478/helm-2019-0010

HELMINTHOLOGIA, 56, 2: 151 – 156, 2019

On two morphologically different cysticercoids of the genus *Eurycestus* (Cestoda: Dilepididae) in *Artemia franciscana* (Arthropoda: Artemiidae) in a hypersaline pond in Dubai, United Arab Emirates*

R. K. SCHUSTER

Central Veterinary Research Laboratory, PO Box 597, Dubai, United Arab Emirates, E-mail: *r.schuster@cvrl.ae*

Article info

Received January 10, 2018
Accepted March 28, 2018

Summary

During a survey on tapeworm larval stages in *Artemia franciscana* from an artificial pond in Dubai, United Arab Emirates, a high prevalence of *Eurycestus avoceti*-like cysticercoids was established. Adult male and female crustaceans showed a prevalence of 61.9 and 62.7 %, respectively. The intensity ranged from one to four and one to three cyst, respectively. Out of 215 examined cysticercoids, 207 specimens had morphological features matching with *E. avoceti*. The flaky structure of the surrounding capsule, the elongated shape of the cysticercoid and the larger number of hooklets on the suckers suggest that the eight further larval cestodes belonged to another species of the genus *Eurycestus*.
Keywords: Brine shrimps; *Artemia franciscana*; cysticercoids; *Eurycestus*; United Arab Emirates

Introduction

Brine shrimps of the genus *Artemia* are aquatic crustaceans that live in hypersaline environments. The genus *Artemia* consists of *A. salina* (Linnaeus, 1758), *A. monica* Verrill, 1869, *A. urmiana* Günther, 1899, *A. franciscana* Kellogg, 1906, *A. persimilis* Piccinelli & Prosdocimi, 1968, *A. sinica* Cai, 1989, *A. tibetiana* Abatzopoulos, Zhang & Sorgeloos, 1998 and parthenogenetic populations called *A. parthenogenetica* (Asem et al. 2010).
Serving as food for flamingos, waders, gulls and ducks, brine shrimps act as intermediate host for a number of avian cestodes of the Hymenolepididae family (*Aploparaksis parafilum* Gasowska, 1932*, Brachiopodataenia gvozdevi* Maksimova, 1988*, Confluaria podicipina*, (Szymanski, 1905)*, Fimbriaruioides tadornae* Maksimova, 1976*, Flamingolepis caroli* (Parona, 1887)*, F. flamingo* Skrjabin 1914 *, F. liguloides* Gervais, 1847, *F. tengizi* Gvozdev & Maksimova, 1968*, Hymenolepis californicus* Young, 1950*, Wardium fusca* (Krabbe, 1869) and *W. stellorae* Deblock, Biguet et Capron, 1960), the Dilepididae family (*Eurycestus avoceti* Clark,1954, *Anomolepis averini* Spassky & Yurpalova, 1967, *Anomotaenia tringae* (Burt,1940) and *A. microphallus* (Krabbe, 1869) and the Progynotaeniidae family *Gynandotaenia stammeri* Fuhrmann, 1936 and *Gynandrotaenia* sp. Redón et al., 2015b – a so far undetermined further species of this genus) (Schuster 2018).
One of the cysticercoids that was found in numerous studies was *E. avoceti* (Table 1). The description of its larval stage is based only on small numbers of cysticercoids. In a recent study on the location of cestode larvae in the body of *A. franciscana*, we examined a larger number of *Eurycestus* cysticercoids showing the existence of two morphologically different types.

Materials and Methods

Collection site of A. franciscana
The Godolphin lakes in the Al Quoz district of Dubai are small ponds of 2.5 and 2.9 ha, respectively and were created as satellite wetland to attract wader birds. A detailed description of this habitat was given by Sivakumar et al. (2018).

* – This paper is dedicated to the 90th birthday of my teacher, Prof. Dr. Dr. h.c. mult. Theodor Hiepe, former director of the Parasitological Institute of the Humboldt-University of Berlin.

Table 1. Frequency of *Eurycestus avoceti* in brine shrimps in different studies.
(F: France, Es: Spain, P: Portugal, AE: United Arab Emirates)

Collection site	Country	Examined host species	Number examined	Prevalence (%)	Average intensity	Reference
Camargue	F	*Artemia* sp.	64.640	0.09	1 – 2	Robert & Gabrion (1991)
Odiel Marsh	Es	*A. parthenogenetica*	3,300	2.5	1 – 3	Georgiev *et al*. (2005)
Odiel Marsh	Es	*A. parthenogenetica*	200	7.0	–	Sanchez *et al*. (2006)
Odiel Marsh	Es	*A. parthenogenetica*	100	17.0	–	Sanchez *et al*. (2007)
Salinas Castro Marim	P	*A. franciscana*	240	4.0	1	Georgiev *et al*. (2007)
Odiel Marsh	Es	*A. parthenogenetica*	200	3.0	1	Georgiev *et al*. (2007)
Salinas de Nuestra	Es	*A. parthenogenetica*	200	7.0	1.14	Georgiev *et al*. (2007)
Salinas Portuguesas	Es	*A. parthenogenetica*	200	30.0	1.15	Georgiev *et al*. (2007)
Salinas de Animas	Es	*A. parthenogenetica*	70	4.3	1	Georgiev *et al*. (2007)
Salinas de St. Barbara	Es	*A. franciscana*	200	2.0	1.4	Georgiev *et al*. (2007)
Salinas de Cerillos	Es	*A. salina*	200	8.0	1	Georgiev *et al*. (2007)
La Mata Lagoon	Es	*A. parthenogenetica*	195	2.6	1	Redon *et al*. (2011)
Odiel Marsh	Es	*A. parthenogenetica*	3,000	2.13	1.08	Sanchez *et al*. (2013)
La Tapa salters	Es	*A. franciscana*	949	2.3	1.09	Georgiev *et al*. (2014)
Ebro delta	Es	*A. franciscana*	9,293	7.6	1.2	Redon *et al*. (2015a)
Ebro delta	Es	*A. franciscana*	487	8.6	1.02	Redon *et al*. (2015b)
Ebro delta	Es	*A. salina*	381	3.9	1	Redon *et al*. (2015b)
Godolphin lakes, Dubai	AE	*A. franciscana*	1,840	4.5	1.1	Sivakumar *et al*. (2018)

Examination of shrimps

Brine shrimps of the species *A. franciscana* were caught by net in the Godolphin lakes in Dubai, in May 2018 for a study of the location of cysticercoids in the body of the shrimps. Shrimps were killed in hot 70 % alcohol and 300 adult specimens were randomly selected. Prior to examination, they were placed for 5 days on a slide in a drop of glycerin and were covered with a cover slip. Glycerin cleared the body of the shrimps and made the parasites visible. Examination for the presence of cysticercoids was carried out at low magnification (40 – 100x). Special attention was paid to cysts surrounded by a brown capsule as well as to those with a transparent capsule and suckers exhibiting hooklets. Measurements of the outer capsule were taken at a magnification of 400x. Shrimps were then dissected and cestode larvae were individually placed into a drop of glycerin. Prior to putting a cover slip, the capsule surrounding the cysticercoid was destroyed by fine preparation needles. At a magnification of 600x, the following parameters of each cysticercoid matching the description of the genus *Eurycestus* were determined: length and width of the cysticercoid, width of the scolex at the level of suckers, length and width of suckers, distribution, number and length of hooklets on suckers, length and width of the rostellum, number and length of rostellar hooks. In order to determine the exact length of sucker hooklets and rostellar hooks as well as the number of rostellar hooks most of the cysticercoids had to be squashed by pressing on the cover slip. Measurements were taken using an OLYMPUS BX51 microscope connected to an OLYMPUS DP27 camera with the software OLYMPUS cellSens Dimension.

Ethical Approval and/or Informed Consent

All applicable national and institutional guidelines for the care and use of animals were followed.

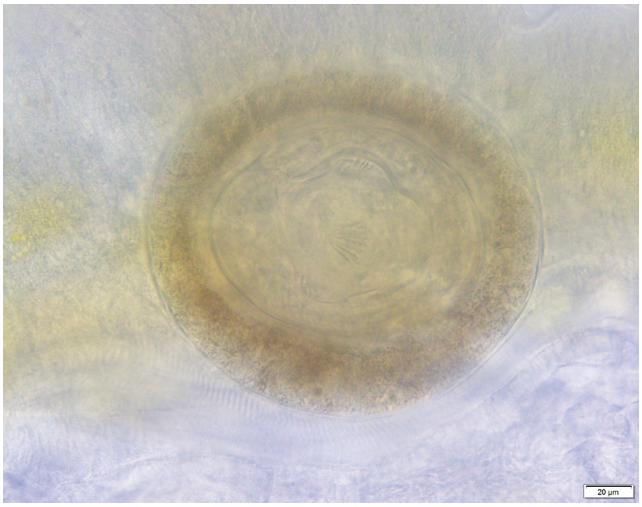

Fig. 1. *Eurycestus avoceti* cysticercoid surrounded by a homogenous brown capsule and found in the thorax of *Artemia franciscana*.

Table 2. Prevalence and burden of *Eurycestus* cysticercoids in *A. franciscana* from Godolphin lakes of Dubai in May 2018.

Sex	Number of		Prevalence (%)	Intensity	
	examined	parasitised		average	range
males	134	83	61.94	1.14	1 – 4
females	166	104	62.65	1.15	1 – 3
total	300	187	62.33	1.15	1 – 4

Results

Of 300 examined *A. franciscana*, 134 were males and 166 were females. A total of 187 (= 62.33 %) of crustacean hosts harbored between one and four *Eurycestus* cysticercoids. There was no sex related difference in prevalence and burden (Table 2). Out of 95 *Eurycestus* cysticercoids in male shrimps, 93 were located in the thorax and each one in the abdomen and phyllopods. Female shrimps contained a total of 120 *Eurycestus* cysticercoids. Of these, 114 were detected in the thorax, four and two were found in head and abdomen, respectively.

The majority (n=207) of the examined 215 cysticercoids was surrounded by a more or less homogenous, transparent to dark

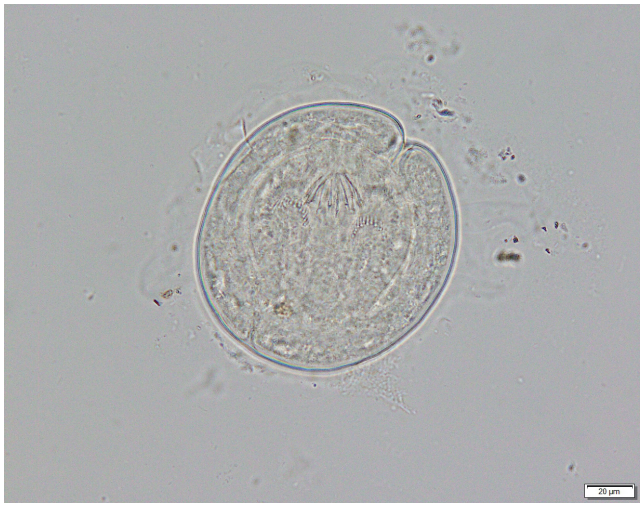

Fig. 2. *Eurycestus avoceti* removed from its capsule.

brown irregularly rounded capsule (Fig 1). The decapsulated cestode larval stages were heart shaped to oval round (Fig 2.) The anterior margin of the oval suckers was furnished with two layers of hooklets in total numbers between 8 and 15. In most of the cases 12 hooklets were counted (Table 3). Their length was 5 – 7 µm. There were 14 to 16 arcuatoid rostellar hooks arranged in two circles. Their length varied between 12 to 16 and 14 to 18 µm, respectively.

Eight other *Eurycestus* cysticercoids were surrounded by an irregularly elongated, flaky and very fragile capsule (Fig. 3). The decapsulated cysticercoids were elongated (Fig. 4) and differed from the above mentioned by a larger number of hooklets on the suckers ranging between 24 and 32 (Table 3). Sixteen arcuatoid rostellar hooks arranged in two circles measured 11 – 14 and 17 – 19 µm, respectively.

Discussion

While previous publications reported prevalence data of *Eurycestus* cysticercoids in *Artemia* spp. between 0.09 and 30.0 % (Table 1), the current study revealed an unusually high prevalence of 62.33 %. The reasons for this might be the small size of the habitat, a large number of birds visiting the ponds and a specific moment in time for the collection when *Artemia* population was in full bloom in the month of May.

The species inventory of the genus *Eurycestus* consists so far of three species. In addition to the description of *E. avoceti* from its final host, the American avocet (*Recurvirostra americana* Gmelin, 1789) by Clark (1954), Burt (1979) described two further species of the genus *Eurycestus* found in the same final host. *E. falciformis* and *E. latissimus* differed from *E. avoceti* in the shape of strobila, size of cirrus sac, number of testes and armature of the cirrus. All three descriptions were based on strobilae without scolex.

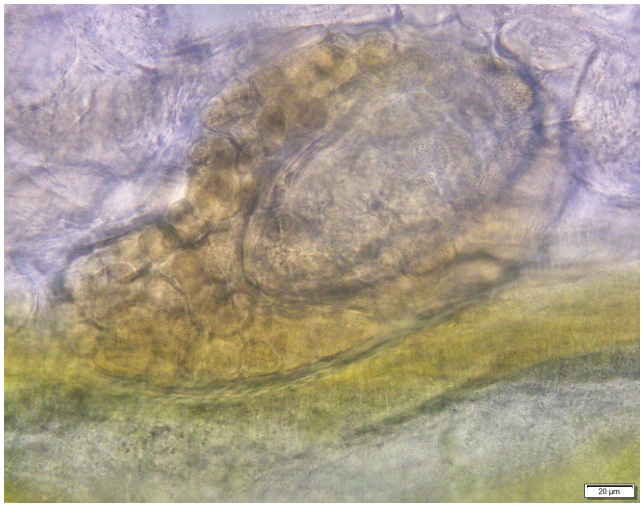

Fig. 3: *Eurycestus* sp. cysticercoid surrounded by a flaky capsule fragile capsule and found in the thorax of *Artemia franciscana.*

In addition to American and pied avocets from Nebraska and France, respectively, *E. avoceti* was found also in black necked stilts *(Himantopus mexicanus* (Müller, 1776) in Texas (Hinojus & Canaris 1988). Maksimova (1991) mentioned greater flamingos (*Phoenicopterus roseus* Pallas, 1811), black-winged stilts (*Himan-*

Table 3. Morphometrical data of two morphologically different *Eurycestus* cysticercoids in *Artemia franciscana* fom Godolphin lakes of Dubai.

Type		Outer capsule		Cysticercoid		Skolex	suckers				Rostellum		Rostellar hooks		
									hooklets				n	small	large
		length	width	length	width	width	length	width	n	length	length	width		length	length
A	average	173.7	152.2	124.1	100.1	63.8	18.6	15.5			58.1	20.1		13.8	16.2
N=207	range	130 – 246	102 – 215	100 – 153	70 – 146	50 – 94	14 – 25	10 – 20	8 – 15	5 – 7	44 – 88	14 – 26	14 – 16	12 – 16	14 – 18
B	average	211.5	123	157.9	90.5	78	22.4	20.3		7	65.8	18.8	16	12	17.8
N=8	range	182 – 245	93 – 160	145 – 186	76 – 113	63 – 91	21 – 24	18 – 22	24 – 30		58 – 78	13 – 21		11 – 14	17 – 19

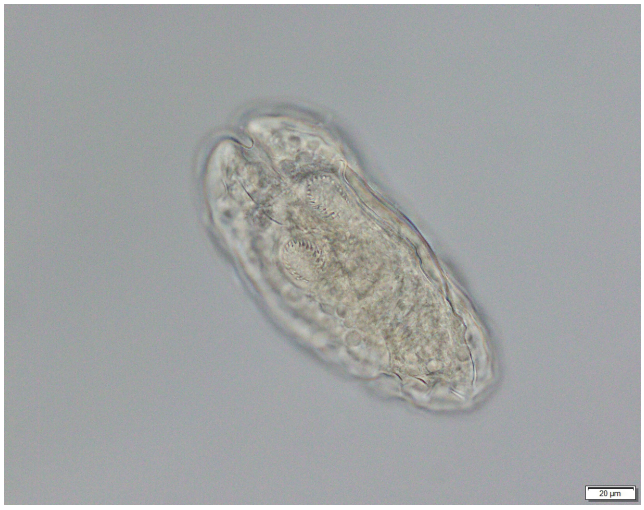

Fig. 4: *Eurycestus* sp. cysticercoid removed from its capsule.

topus himantopus Linnaeus, 1758) and slender-billed gulls (*Chroicocephalus genei* Breme, 1839*)* as final hosts in Kazakhstan. This wide spectrum of phylogenetically distant hosts suggests that also other birds that feed on brine shrimps might play a role in the *Eurycestus* life cycle.

The assignment of *E. avoceti* to the Anoplocephalidae family by Yamaguti (1959) was proven incorrect after Baer (1968) found whole cestode including scolex in avocets[a] in Carmargue, France and published a redescription of the species. The width of the extremely small scolex varied between only 45 and 53 µm and could reach 61 µm when the rostellum (52 µm long and 20 µm in diameter) is retracted. There were 14 to 16 rostellar hooks in a length of 14 to 16 µm, arranged in two circles. Suckers bore 10 to 14 hooklets in a length of 5 – 6 µm. Baer (1968) allocated *E. avoceti* to the Dilepididae family. Based on the morphology of the scolex, Georgiev et al. (2005) considered cysticercoids found in *A. parthenogenetica* in the Odiel Marshes in Spain, the larval stage of *E. avoceti*. The majority of cysticercoids found in the current study (type A in Table 3) seem to belong to the same species, *E. avoceti*. Eight other cysticercoids in the recent study (type B, in Table 3) however, showed striking differences in the structure of the capsule, surrounding the larval stage and in the shape of the cysticercoid. A further difference was the number and the distribution of the hooklets on the suckers. Already Gabrion & Mac Donald (1980) when examining *Artemia* sp. from Camarge, gave a description of an elongated cysticercoid (170 x 90 µm) suckers of which were furnished with 30 to 32 hooklets covering their anterior and lateral rims. Other parameters (number and size of rostellar hooks) did not differ significantly from above mentioned *E. avoceti*. Robert & Gabrion (1991) examined a total of 64,604 *Artemia* sp. in the same habitat and found among others, a total of 59 cysticercoids matching the description by Gabrion et Mac Donald (1980). In a survey on cestode larval stages in *A. salina* from Tengiz lake in Kazakhstan, Maksimova (1991) described elongated cysticercoids measuring 140 – 190 x 100 – 130 µm being surrounded by a dark brown but very fragile capsule of 180 – 320 x 150 – 220 µm in size. The scolex measured 45 – 76 x 42 – 71 µm and had four armed suckers with 30 to 32 hooklets arranged in two rows on their anterior and lateral margins. The rostellum had a length of 50 – 60 µm and a width of 17 – 22 µm. It was armed with 16 hooks arranged in two rows. Larger hooks measured 16 – 18 µm, smaller ones 10 – 12 µm. The prevalence of these cysticercoids in *A. salina* in lake Tengiz was low and varied between 0.03 and 0.5 %. Referring to Gabrion et Mac Donald (1980) the author believed that this was *E. avoceti*.

Based on the appearance of the surrounding capsule and on the morphology of the cysticercoid, it can be concluded that *A. franciscana* in the Godolphin lakes of Dubai were infected with two different *Eurycestus* species one of which type A can be affiliated with *E. eurycestus*. The second species (type B) comparable to those found *Artemia* sp. in Carmargue and in *A. salina* in Tengiz lake remains unnamed until the adult cestode is described.

Conflict of Interest

There is no conflict of interests.

References

Asem, A., Rastegar-Pouyani, N., De Los Ríos-Escalante P. (2010) The genus *Artemia* Leach, 1819 (Crustacea: Branchiopoda). I. True and false taxonomical descriptions. *Lat. Am. J. Aquat. Res.*, 38(3): 501 – 506. DOI: 10.3856/vol38-issue3-fulltext-14

Baer J.G. (1968): *Eurycestus avoceti* Clark, 1954 (Cestode cyclophyllidien) parasite de l' avocette en Camargue. [*Eurycestus avoceti* Clark, 1954 (Cestoda; Cyclophyllidea) a parasite of the avocet in the Camargue]. *Vie et Milieu,* 19: 189 – 198. (In French)

Burt, D.R.R. (1979): New cestodes of the genus *Eurycestus* Clark 1954 from the avocet *Recurvirostra americana* Gmelin 1788. *Zool. J. Linn. Soc.,* 65: 71 – 82

Clark, D.T. (1954): A new cyclophyllidian cestode from the avocet. *J. Parasitol.* 40: 340 – 346.

Gabrion, C., Mac Donald, G. (1980): *Artemia* sp. (Crustacé, Anostracé), hôte intermédiaire d' *Eurycestus avoceti* Clarc, 1954 parasite de l'avocette en Camargue. [*Artemia* sp. (Crustacea, Anostraca) the intermediate host of *Eurycestus avoceti* Clarc, 1954 a parasite of the avocet in the Camargue.] *Ann. Parasitol.,* 55 (3): 327 – 331 (In French)

Georgiev, B.B., Angelov, A., Vasilieva, G.P., Sanchev, M.I., Hortas, F., Mutavchiev, Y., Pankov, P., Green, A.J. (2014): Larval helminths in the invasive American brine shrimp *Artemia franciscana* throughout its annual cycle. *Acta Parasitol.,*59 (3): 380 – 389. DOI: 10.2478/s11686-014-0255-x

Georgiev, B.B., Sanchez, M.I., Green, A.J., Nikolov, P.N., Vasilieva, G.P., Mavrodieva, R.S. (2005): Cestodes from *Artemia parthenogenetica* (Crustacea, Branchiopoda) in the Odiel Marshes, Spain: a systematic survey of cysticercoids. *Acta Parasitol.* 50 (2): 105 – 117

a – The bird species was not mentioned in the paper but most probably it was the pied avocet (*R. avosetta*).

Georgiev, B.B., Sanchez M.I., Nikolov, P.N. Vasilieva, G.P., Green, A.J. (2007): Cestode parasitism in invasive and native brine shrimps (*Artemia* spp.) as a possible factor promoting the rapid invasion of *A. franciscana* in the Mediterranian region. *Parasitol. Res.* 101 (6): 1664 – 1655. DOI: 10.1007/s00436-007-0708-3

Hinojus, J.G., Canaris, A.G. (1988): Metazoon parasites of *Himantopus mexicanus* Muller (aves) from Southwestern Texas, with a checklist of helminth parasites from North America. *J. Parasitol.* 74 (2): 326 – 331

Maksimova, A.P. (1991): K ekologii i biologii *Eurycestus avoceti* (Cestoda: Dilepididae). [On ecology and biology of *Eurycestus avoceti* (Cestoda: Dilepididae]. Parazitologiya 25: 73 – 76

Robert, F., Gabrion, C (1991) Cestodoses de l'avifanune camarguaise. Rôle d'Artemia (Crustacea, Anostraca) et strategies de rencontre hôte-parasite. [Cestodes of birds in Camargue. Importance of Artemia (Crustacea, Anostraca) and meeting strategies between hosts and parasites] *Ann. Parasitol. Hum. Comp.* 66 (5): 226 – 236 (In French)

Redon, S., Amat, F., Hontoria, F., Vasilieva, G., Nikolov, P.N., Georgiev, B.B. (2011): Participation of metanaulplii and juvenile individuals of *Artemia parthenogenetica* (Branchiopoda) in the circulation of avian cestodes. *Parasitol. Res*. 108(4): 905 – 912. DOI 10.1007/s00436-010-2132-3

Redón, S., Amat, F., Sánchez, M.I., Green, A.J. (2015a): Comparing cestode infections and their consequences for host fitness in two sexual branchiopods: alien *Artemia franciscana* and native *A. salina* from syntopic-populations. *Peer J,* 3:e1073; DOI: 10.7717/peerj.1073

Redón, S., Green, A.J., Georgiev, B.B., Vasileva, G.P., Amat, F. (2015b): Influence of developmental stage and sex on infection of the American brine shrimp *Artemia franciscana* Kellogg, 1906 by avian cestodes in Ebro Delta salterns, Spain. *Aquat. Invasions* 10 (4), 415 – 423. DOI: 10.3391/ai.2015.10.4.0

Sanchez M.I., Georgiev, B.B., Green, A.J. (2007): Avian cestodes affect the behavior of their intermediate host *Artemia parthenogenetica*: An experimental study. *Behav. Process.* 74: 293 – 299. DOI: 10.1016/j.beproc.2006.11.002

Sanchez M.I., Georgiev, B.B., Nikolov, P.N. Vasilieva, G.P., Green, A.J. (2006): Red and transparent brine shrimps (*Artemia parthenogenetica*): a comparative study of their cestode infections. *Parasitol. Res.* 100 (1): 111 – 114. DOI: 10.1007/s00436-006-0248-2

Sanchez M.I., Nikolov, P.N., Georgieva, D.D., Georgiev, B.B., Vasilieva, G.P., Pankov, P., Paracuellos, M., Lafferty K.D., Green, A.J. (2013): High prevalence of cestodes in *Artemia* spp. throughout the annual cycle: relationship with abundance of avian final hosts. *Parasitol. Res.* 112 (5): 1913 – 1923. DOI: 10.1007/s00436-013-3347-x.

Schuster R.K. (2018): The role of *Artemia* spp. (Branchiopoda: Artemiidae) as intermediate hosts for avian cestodes. Environ Anal Eco stud. 1(4). EAES.000518.2018. DOI: 10.31031/EAES.2018.01.000518

Sivakumar, S., Hyland, K. Schuster R.K. (2018): Tapeworm larvae in *Artemia franciscana* (Crustacea: Anacostra) in the Goldolphin lakes of Dubai (United Arab Emirates) throughout an annual cycle. *J. Helminthol*. 1 – 7. DOI: 10.1017/S0022149X18000913

Yamaguti S. (1959) *Systema helminthum 2. The cestodes of vertebrates.* New York, London, Interscience Publishers, 860 pp.

sciendo

DOI 10.2478/helm-2019-0012

HELMINTHOLOGIA, 56, 2: 157 – 167, 2019

First report on mermithid parasitism (Enoplea: Mermithidae) in a Southeast Asian spider (Araneae: Araneidae)

O. KOŠULIČ[1], Š. MAŠOVÁ[2]*

[1]Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 3, Brno 613 00, Czech Republic; [2]*Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, Brno 611 37, Czech Republic, E-mail: *masova.sarka@gmail.com*

Article info

Received February 1, 2019
Accepted February 14, 2019

Summary

Details about the record of a juvenile mermithid roundworm parasitizing the bark spider *Caerostris sumatrana* Strand, 1915 from Thailand are presented. The morphology and ecology of both organisms is discussed. Morphological features suggest this juvenile nematode belongs to the genus cf. *Aranimermis*. Due to the subadult stage of parasite, identification to species-level was not possible. This first report of a nematode infection in *C. sumatrana* with several recent findings from other studies significantly adds to the current inventory of mermithids parasitizing spiders. Moreover, our finding is among the first record of this host-parasite interaction from Southeast Asia.
Keywords: *Agamomermis*; *Aranimermis*; *Caerostris sumatrana*; orb-web spider; parasitoid; Thailand

Introduction

Spiders are top-predators in arthropod communities playing an important role in insect reduction (Marc *et al*., 1999; Michalko & Pekár, 2015; Michalko *et al*., 2019). However, there are many groups of invertebrates that also feed on spiders. In this respect, parasites and parasitoids are among the very important group of natural enemies of spiders. Recently, records of the parasites and parasitoids of spiders are becoming more frequent and many authors have described occurrences of parasitisation by hymenopterous, dipterous, and neuropterous parasitoids (e.g. Allard & Robertson, 2003; Finch, 2005; Takasuka *et al*., 2017). However, very little is known about the relevance of nematode parasitism in spiders (Poinar, 1987; Penney & Bennett, 2006). These findings are rare and the parasite is usually not identified to species or even genus due to the difficulties in identification which require fully mature adult specimens (Poinar, 1987; Penney & Bennett, 2006). All of known reports of natural nematode parasitism in spiders belong to the group of parasitoids from the family Mermithidae (order Mermithida), which generally parasitize on invertebrates only (Poinar, 1987; Meyer, 2014). Apart from spiders, mermithids can also be found parasitizing in mosquitoes, grasshoppers, butterflies, damselflies or cockroaches (Meyer, 2014). However, for many mermithids, the host species is still unknown (Nickle, 1972).

Up to now, only the following mermithid genera have been recorded to parasite on spiders: *Mermis* Dujardin, 1845; *Hexamermis* Steiner, 1924; *Agamermis* Cobb, Steiner & Christie, 1923; *Heydenius* Taylor, 1935 and *Arachnomermis* Rubtsov, 1978 (Poinar, 1985; Poinar, 2000; Poinar, 2012). Further, Poinar and Welch (1981) erected a collective genus *Agamomermis* for all identifications based on juvenile material unsuitable for species identification (Poinar, 1987; Allard and Robertson, 2003). Afterwards, Poinar and Benton (1986) and Poinar & Early (1990) provided the first descriptions based on adult specimens of mermithids and erected the genus *Aranimermis* with a general characterization of this distinctive genus that is trophically specialized on spiders.

Parasitism by a mermithid is fatal to the host (Nikdel *et al*., 2011; Poinar, 1983). The life cycle including five stages is described in Poinar (1983): egg, second stage juvenile (preparasitic infective juvenile), parasitic third stage juvenile, mature third stage

* – corresponding author

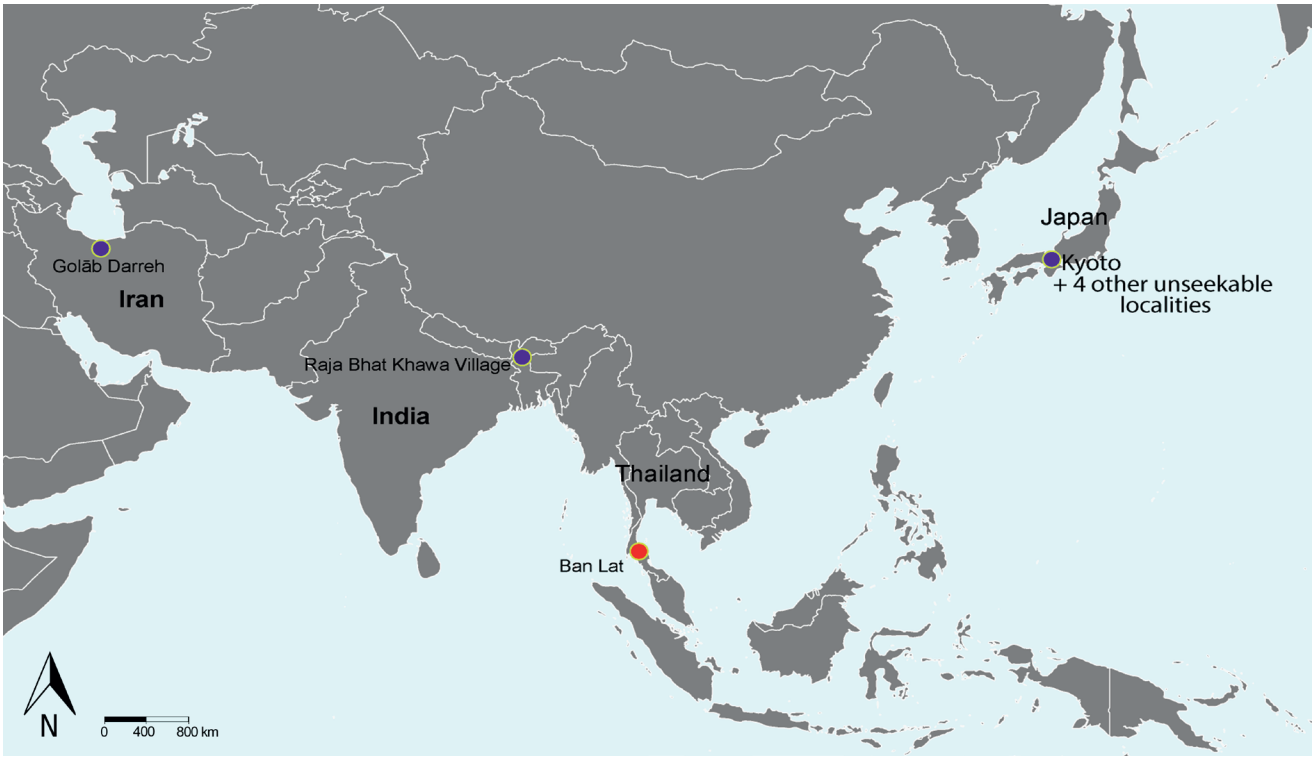

Fig. 1. Distribution map of mermithids parasiting in spiders throughout the whole area of Asia. All seven records (blue circles) from Asia are taken from the following literature: Okochi (1969); Matsuda (1999); Iida and Hasegawa (2003); Zamani (2014); Ranade and Prakash (2016). Our finding is marked by red coloured circle (for locality description see Material and Methods).

postparasitic juvenile, two molts into adult. These parasitoids have parenteral intake of nutrition from the host tissues and haemolymph which may strongly influence the physiological condition of the host since the first instars of parasite development (Nikdel *et al*., 2011). Afterwards, during the emergence of the (still juvenile) parasite, the mermithid nematode kills its host (Poinar, 1987). In this postparasitic free-living stage, the parasite does not feed anymore and only needs a suitable habitat to mature.

Spiders from a variety of functional guilds can be infected with mermithids. An important factor for the completion of these nematodes' life cycle is the presence of water, wet soil, or mud where they can mature after emergence from the spider host (Poinar, 1987; Meyer, 2014). Regardless, the ground dwelling and active hunter spiders are parasitized more often in comparison with orb web or space web spiders (e.g. Penney & Bennett, 2006; Zamani, 2014). Usually, spiders become infected by ingesting a paratenic host or directly by being penetrated by an infective larva that has recently hatched out from eggs (Penney & Bennett, 2006). Infected spiders may show symptoms such as an enlarged and deformed abdomen, deformed copulation structures, or deformed legs. Moreover, they may show changes in behaviour such as lethargy, slower reaction times to predators, and a tendency to move towards water (Poinar, 1985; Pizzi, 2009; Meyer, 2014).

The purpose of the present paper is to report the first finding of a mermithid nematode, which parasites in the *Caerostris sumatrana* Strand, 1915 from the region of Southeast Asia along with ecological notes on the distributions of spider mermithids. To date, there are no records of endoparasitism by nematodes in orb-web spiders from the Asian region and furthermore, there are not even reports of mermithids in any groups of spiders from Southeast Asia. The postparasitic juvenile stage is described and its characteristics are illustrated. The list of records of mermithid infections made by Poinar (1987) and Penney & Bennett (2006) is updated according to the recent findings (Rodrigues *et al*., 2005; Meyer, 2014; Zamani, 2014; Ranade & Prakash, 2016) and amended with the location and region of the host-parasite finding.

Materials and methods

The two *Caerostris* spiders were collected on the edge of a dry evergreen forest in the Phethaburi province of Ban Lat district in the central part of Thailand (12°96'85"N, 99°66'53"E; Fig. 1). The hilly landscape area of the locality was at an altitude of 300-400 m a.s.l. The area from which the parasitized spider was collected was sheltered by a dense canopy of dry evergreen forest with presence of river streams and small patches of disturbed and logged forest stands. Spiders were sampled during rainy season (July 2014), thus the environment in the location was very humid and influenced by short-term heavy rains. Specimens were collected at night by visually searching and removing individuals directly from

Table 1. Summary of all records of mermithids from spiders around the world.
* Unknown country, however the geographic region can be determined by the distribution of the spider host. Spider families alphabetically arranged.

Family	Host of mermithid	Hunting guild	Mermithid	Country	Continent	Reference
Agelenidae	*Agelenopsis oregonensis* Chamberlin & Ivie 1935	Sheet web weavers	*species inquirendae*	USA	North America	in Poinar (1987)
Amaurobiidae	*Eurocoelotes inermis* (L. Koch 1855)	Sheet web weavers	*species inquirendae*	Germany	Europe	in Poinar (1987)
Antrodiaetidae	*Atypoides riversi* O.P.-Cambridge 1833	Sensing web weavers	*species inquirendae*	USA	North America	in Poinar (1987)
Anyphaenidae	*Wulfila albens* (Hentz, 1847)	Other hunters	*Aranimermis aptispicula* Poinar & Benton, 1986	USA	North America	in Poinar (1987)
Araneidae	*Aculepeira ceropegia* (Walckenaer 1802)	Orb web weavers	*species inquirendae*	UK	Europe	in Poinar (1987)
Araneidae	*Araneus diadematus* Clerck 1757	Orb web weavers	*species inquirendae*	UK	Europe	in Poinar (1987)
Araneidae	*Verrucosa arenata* (Walckenaer 1842)	Orb web weavers	*Aranimermis aptispicula* Poinar & Benton, 1986	USA	North America	in Poinar (1987)
Ctenidae	*Leptoctenus byrrhus* Simon 1888	Other hunters	*species inquirendae*	UK	Europe	Poinar (2000) in Penney & Bennet (2006)
Cybaeidae	*Argyroneta aquatica* (Clerck 1757)	Sheet web weavers	*species inquirendae*	UK	Europe	in Poinar (1987)
Gnaphosidae	*Cesonia bilineata* (Hentz 1847)	Ground hunters	*Aranimermis aptispicula* Poinar & Benton, 1986	USA	North America	in Poinar (1987)
Gnaphosidae	*Gnaphosa lucifuga* (Walckenaer 1802)	Ground hunters	*species inquirendae*	Germany	Europe	in Poinar (1987)
Hexathelidae	*Porrhothele antipodiana* (Walckenaer 1837)	Sheet web weavers	*Aranimermis giganteus* Poinar & Early, 1990	New Zealand	Australia	Poinar & Early (1990) in Penney & Bennet (2006)
Idiopidae	*Misgolas borealis* (Forster 1968)	Sensing web weavers	*Aranimermis giganteus* Poinar & Early, 1990	New Zealand	Australia	Poinar & Early (1990) in Penney & Bennet (2006)
Linyphiidae	*Micryphantes bicuspidatus* C.L. Koch 1838	Sheet web weavers	*species inquirendae*	*	Europe	in Poinar (1987)
Lycosidae	*Alopecosa inquilina* (Clerck 1757)	Ground hunters	*species inquirendae*	*	Europe	in Poinar (1987)
Lycosidae	*Alopecosa trabalis* (Clerck 1757)	Ground hunters	*species inquirendae*	*	Europe	in Poinar (1987)
Lycosidae	*Arctosa alpigena* (Doleschall 1852)	Ground hunters	*species inquirendae*	UK	Europe	Poinar (2000) in Penney & Bennet (2006)

Lycosidae	*Geolycosa patellonigra* Wallace 1942	Ground hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Lycosidae	*Hygrolycosa rubrofasciata* (Ohlert 1865)	Ground hunters	*species inquirendae*	Finland	Europe	Ahtiainen et al. (2004) in Penney & Bennet (2006)
Lycosidae	*Pardosa agrestis* (Westring 1861)	Ground hunters	*species inquirendae*	*	Europe	in Poinar (1987)
Lycosidae	*Pardosa amentata* (Clerck 1757)	Ground hunters	*species inquirendae*	*	Europe	in Poinar (1987)
Lycosidae	*Pardosa furcifera* (Thorell 1875)	Ground hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Lycosidae	*Pardosa glacialis* (Thorell 1872)	Ground hunters	*species inquirendae*	Greenland	North America	in Poinar (1987)
Lycosidae	*Pardosa hortensis* (Thorell 1872)	Ground hunters	*species inquirendae*	*	Europe	in Poinar (1987)
Lycosidae	*Pardosa lugubris* (Walckenaer 1802)	Ground hunters	*species inquirendae*	*	Europe	in Poinar (1987)
Lycosidae	*Pardosa milvina* (Hentz 1844)	Ground hunters	*species inquirendae*	Canada	North America	in Poinar (1987)
Lycosidae	*Pardosa palustris* (Linneaus 1758)	Ground hunters	*species inquirendae*	*	Europe	in Poinar (1987)
Lycosidae	*Pardosa pseudoannulata* (Boesenberg & Strand 1906)	Ground hunters	*species inquirendae*	Japan	Asia	Iida & Hasegawa (2003) in Penney & Bennet (2006)
Lycosidae	*Pardosa riparia* (C.L. Koch 1833)	Ground hunters	*species inquirendae*	*	Europe	in Poinar (1987)
Lycosidae	*Pardosa sphagnicola* (Dahl 1908)	Ground hunters	*species inquirendae*	*	Europe	in Poinar (1987)
Lycosidae	*Pardosa suwai* Tanaka 1985	Ground hunters	*species inquirendae*	Japan	Asia	Matsuda (1999) in Penney & Bennet (2006)
Lycosidae	*Pardosa vancouveri* Emerton 1917	Ground hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Lycosidae	*Prolycosides amblygyna* (Mello-Leitão, 1942)	Ground hunters	*species inquirendae*	Peru	South America	in Rodrigues et al. (2005)
Lycosidae	*Rabidosa rabida* (Walckenaer 1837)	Ground hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Lycosidae	*Schizocosa saltatrix* (Hentz 1844)	Ground hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Lycosidae	*Sosippus floridanus* Simon 1898	Ground hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Lycosidae	*Trochosa terricola*Thorell, 1856	Ground hunters	*species inquirendae*	Germany	Europe	in Meyer (2014)
Nemesiidae	*Stanwellia kaituna* (Forster 1968)	Sensing web weavers	*Aranimermis giganteus* Poinar & Early, 1990	New Zealand	Australia	Poinar & Early (1990) in Penney & Bennet (2006)
Oxyopidae	*Oxyopes sertatus* L. Koch 1877	Other hunters	*species inquirendae*	Japan	Asia	Okochi (1969) in Penney & Bennet (2006)
Oxyopidae	*Peucetia viridans* (Hentz 1832)	Other hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Philodromidae	*Tibellus oblongus* (Walckenaer 1802)	Other hunters	*species inquirendae*	Germany	Europe	in Poinar (1987)
Salticidae	*Frigga sp.* (C. L. Koch, 1850)	Other hunters	*species inquirendae*	Brazil	South America	in Rodrigues et al. (2005)

Salticidae	*Habronattus signatus* (Banks 1900)	Other hunters	*species inquirendae*	Hawaii (USA)	Oceania	Vandergast & Roderick (2003) in Penney & Bennet (2006)
Salticidae	*Myrmarachne formicaria* (De Geer 1778)	Other hunters	*species inquirendae*	*	Europe	in Poinar (1987)
Salticidae	*Phidippus borealis* Banks 1895	Other hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Salticidae	*Phidippus clarus* Keyserling 1885	Other hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Salticidae	*Phidippus johnsoni* (Peckham Peckham 1883)	Other hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Salticidae	*Phidippus putnami* (Peckham Peckham 1883)	Other hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Salticidae	*Sitticus floricola palustris* (Peckham & Peckham 1883)	Other hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Salticidae	*Thiodina sp.* (Simon, 1900)	Other hunters	*species inquirendae*	Brazil	South America	in Rodrigues et al. (2005)
Sparrassidae	*Heteropoda venatoria* (Linnaeus, 1767)	Other hunters	*species inquirendae*	India	Asia	in Ranade & Prakash (2016)
Stiphidiidae	*Cambridgea foliata* (L. Koch 1872)	Sheet web weavers	*species inquirendae*	New Zealand	Australia	in Poinar (1987)
Tetragnathidae	*Tetragnatha anuenue* Gillespie 2002	Orb web weavers	*species inquirendae*	Hawaii (USA)	Oceania	Vandergast & Roderick (2003) in Penney & Bennet (2006)
Tetragnathidae	*Tetragnatha brevignatha* Gillespie 1991	Orb web weavers	*species inquirendae*	Hawaii (USA)	Oceania	Vandergast & Roderick (2003) in Penney & Bennet (2006)
Tetragnathidae	*Tetragnatha praedonia* L. Koch 1878	Orb web weavers	*species inquirendae*	Japan	Asia	Okochi (1969) in Penney & Bennet (2006)
Tetragnathidae	*Tetragnatha quasimodo* Gillespie 1991	Orb web weavers	*species inquirendae*	Hawaii (USA)	Oceania	Vandergast & Roderick (2003) in Penney & Bennet (2006)
Theridiidae	*Enoplognatha ovata* (Clerck 1757)	Space web weavers	*species inquirendae*	*	Europe	in Poinar (1987)
Thomisidae	*Diaea dorsata* (Fabricius 1777)	Ambush hunters	*species inquirendae*	*	Europe	in Poinar (1987)

Thomisidae	*Misumenops tricuspidatus* (Fabricius 1775)	Ambush hunters	*species inquirendae*	Japan	Asia	Okochi (1969) in Penney & Bennet (2006)
Thomisidae	*Xysticus deichmanni* Soerensen 1898	Ambush hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Thomisidae	*Xysticus durus* (Soerensen 1898)	Ambush hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Thomisidae	*Xysticus funestus* Keyserling 1880	Ambush hunters	*species inquirendae*	USA	North America	in Poinar (1987)
Thomisidae	*Heriaeus spinipalpus* Loerbroks 1983	Ambush hunters	*species inquirendae*	Iran	Asia	in Zamani (2014)
Zoridae	*Zora maculosa* Roewer 1951	Ground hunters	*species inquirendae*	*	Europe	in Poinar (1987)

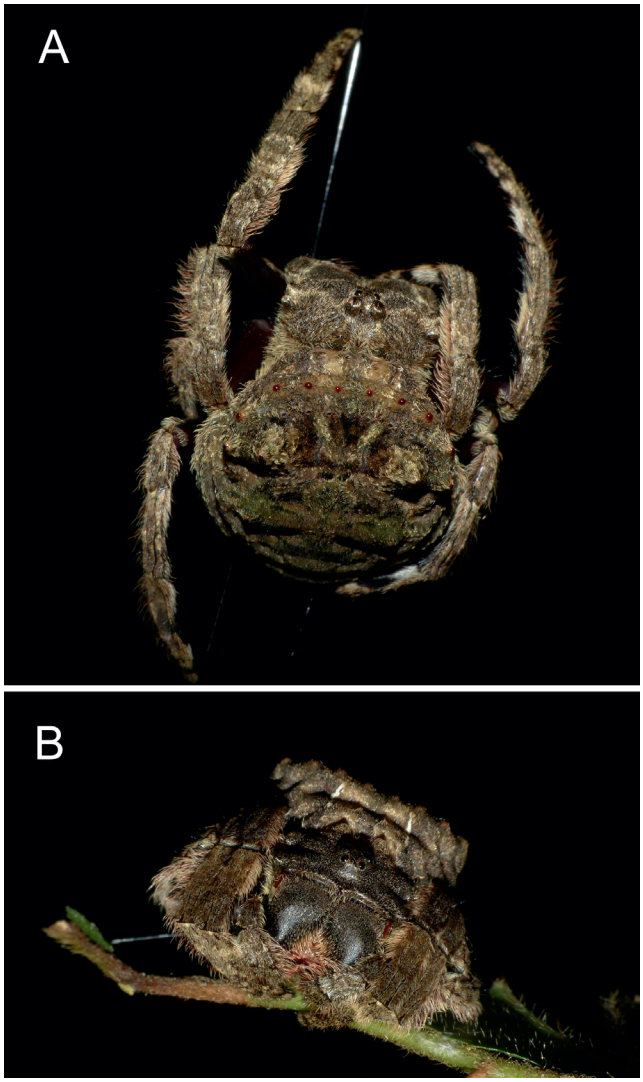

Fig. 2. The parasited spider *Caerostris sumatrana* in an orb-web before collecting. **A:** Overal view of spider body without any visible deformations caused by its parasitoid. **B:** Front view of the same specimen.

the round web. Both females were identified according to available literature (Murphy & Murphy, 2000) as orb-web spiders, *Caerostris sumatrana*. After collection, the obtained material was kept alive in 60 ml individual plastic jars.

Parasite emergence from the host started after eight hours of live spider captivity. No freeliving post-parasites were found. After hatching the post-parasitic juvenile as well as the dead spider host were fixed in 70 % ethanol. For light microscopy examination the nematode was transferred gradually into solution of glycerine and water (2:1) before mounting on microscope slides. The examination of the parasite was performed by using an Olympus BX 50 microscope equipped with differential interference contrast (DIC) optics, digital image analysis system (Motion Stream), and a drawing attachment. Graphic processing of images were maintained and modified in Adobe Illustrator CS3 and Photoshop CS4.

The spider and the mermithid nematode have been both deposited in the collection of the Faculty of Forestry and Wood Technology of Mendel University in Brno (voucher number Mendelu-FF-1545).

Ethical Approval and/or Informed Consent

The conducted research is not related to either human or animals use. Informed consent has been obtained from all individuals included in this study.

Results

In total, two adult females of *C. sumatrana* (Fig. 2A, B) were found at the locality and were found very close to each other (15 metres). No significant differences in body size or the constitution of both spider samples were found (total length 15.3 mm, opisthosoma 6.2 mm long and 5.1 mm wide). One sample of *C. sumatrana* was infected by a nematode which emerged from the host after eight hours of the spider being kept in captivity in a 60 ml plastic jar. Second specimen of spider was kept in captivity for several weeks and no parasitism evidence was found. Therefore, only one specimen of postparasitic juvenile was obtained and measured.

The specimen was brownish in colour (Fig. 3), slightly transparent at the tapered rounded ends (Fig. 4). The mermithid was extremely long in respect to its spider host, it was 28.1 cm in length and a maximum of 1.2 mm wide. It was determined as a collective genus *Agamomermis* Poinar et Welch, 1981, because identification to species level based on such juvenile stage was not possible. However, the morphological features of the examined post parasitic juvenile, including the presence of six cephalic papillae in one plane, amphids located near the lateral cephalic papillae, terminal mouth opening, the lack of lateral lip papillae, and the pointed appendage at the end of the tail (Fig. 4-5), were consistent with those of post parasitic juveniles from the genus *Aranimermis* Poinar & Beton, 1986, a species specialized on parasiting in spiders. Based on these charactecters, the specimen was classified as genus cf. *Aranimermis*.

Discussion

Spider mermithids are recorded from various parts of the world (Poinar, 1987; Penney & Bennet, 2006; Zamani, 2014), however their findings are usually rare comprising mainly juvenile specimens ineligible for species designations. This is because of their complex life style and development which in natural conditions usually enables only accidental discoveries (Poinar, 1985). In general, there are two ways in which the spider parasiting mermithids can develope: (i) direct life cycle, that includes the direct penetration of the parasite into the host and (ii) the indirect life cycle in which the mermithid enters the body of the spider by infestation of a paratenic host which are most often larvae of various aquatic insects (Poinar & Early, 1990; Zamani, 2014). After the emergence

Fig. 3. The host *Caerostris sumatrana* Strand, 1915 and the live postparasitic juvenile mermithid.
A: Nematode and its killed host after emerging. **B:** Different positions of the mermithid in motion.

from their host, postparasitic juvenile mermithids needs to mature in soil or freshwater (Poinar & Early, 1990). Most of the records are provided from this life stage (individuals directly emerging from the spiders), therefore these specimens lack species characteristics and it is only possible to identify them to the family or genus level. According to Poinar & Welch (1981), the juvenile specimens can only be identified to a collective genus, *Agamomermis*. However, Poinar & Benton (1986) raised a new genus, *Aranimermis,* which includes large and massive species that are specialized on parasitizing in spiders and share the same genus characteristics as our studied sample.

Due to the largeness of some mermithids, parasited spiders often show external morphological abnormalities (Poinar, 1983), e.g. greatly enlarged opisthosoma, an altered epigynum, malformed palpi, shorter and thicker legs, and poorly developed or absent male secondary sexual characteristics (Holm, 1941; Leech, 1966). Nevertheless, our host did not have any visible changes and it looked the same as the other non-infected specimen which was also collected. Meyer (2004) and Rodrigues *et al*. (2005) report that infected spiders did not pose any significant changes in morphology, except for swollen abdomens. Therefore, the absence of any significant morphological changes in our sampled spiders (infected and uninfected) is very surprising, especially as the mermithid parasite reached 28 cm in length. This may be due to the overall massive body-constitution of *Caerostris* spiders. The spiders in which Poinar (1987) reports abnormalities come from the Lycosidae, Salticidae and Thomisidae families which are much smaller than our *Caerostris* spider specimen. In our case, internal organs and morphology was not studied, however, we assume severe damage to internal body parts of the spider as the spider specimen died immediately after parasite emergence. In general, the effect of the parasitoid on host physiological appearance re-

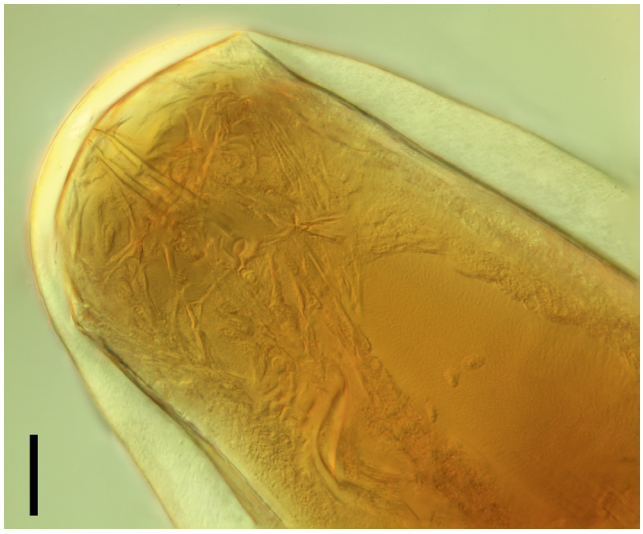

Fig. 4. Light microscopy micrograph (Nomarski contrast, superposition of 11 photos) of the cephalic region of the postparasitic juvenile mermithid from bark spider, *Caerostris sumatrana* Strand, 1915. Scale bar: 50 µm.

quires more research before we can fully understand the overall effect of mermithid parasitoids on the spider´s body and behaviour. Meyer (2014) points out that the infection rate of spiders by mermithids is higher in spider populations located around habitats suitable for parasite maturation, such as wetlands and freshwater streams, which in addition usually support an increased abundance of aquatic insects which serve as important paratenic hosts. Indeed, our finding has been conducted near a water stream and during the tropical wet season that is characterized by frequent rainfalls and very high humidity. We suppose that this season is suitable for the final development and maturation of mermithid parasites as the dry season in tropical lowland forests may not be favourable due to the high drought and lower water level in freshwater streams which also is likely to negatively affect paratenic insect hosts (Murphy & Lugo, 1986). Poinar (1985) point out that most spiders found parasitized would have an opportunity to feed on adult insects which possess an aquatic larval stage. We can support this hypothesis as we have found many prey remains of Trichoptera, Chironomidae and Culicidae in the spider host web. According to Penney & Bennet (2006) and Meyer (2014) the infection rate in some spider populations may reach more than 8 % and is recorded from all trophic guilds of spiders with different hunting strategies (Cardoso *et al*. 2011). This is probably because of the indirect development of mermithids that use insects as paratenic hosts, which are the consumed by various hunting guilds of spiders. However, it seems that the highest prevalence of infection has been usually found in actively hunting spiders such as ground and other hunters from Lycosidae and Salticidae families (Iida & Hasegawa 2003; Penney & Bennet 2006; see updated Table 1). On the other hand, parasitisms in orb-web spiders from Araneidae family, which includes *Caerostris sumatrana,* are known worldwide only from three species reported to date back more than 30 years ago (Poinar, 1987): *Aculepeira ceropegia* (Walckenaer 1802), *Araneus diadematus* Clerck 1757 and *Verrucosa arenata* (Walcken-

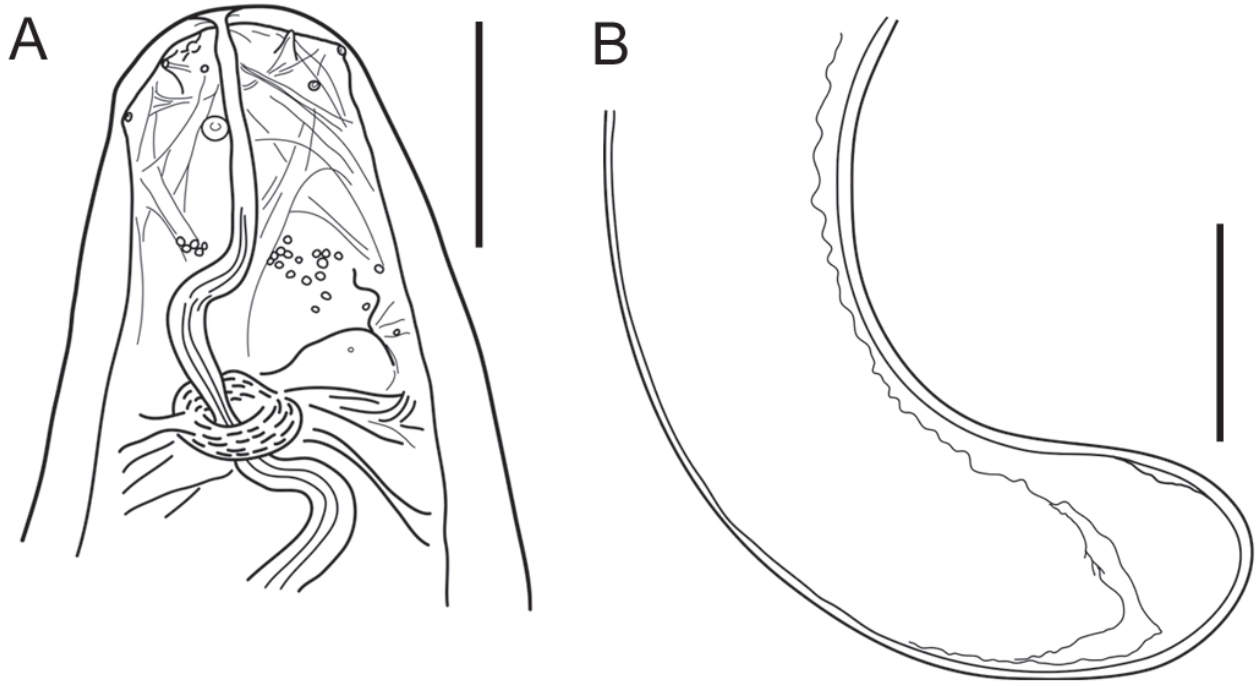

Fig. 5. Line drawings of postparasitic juvenile mermithid from *Caerostris sumatrana* Strand, 1915.
A: Head region, lateral view. **B:** Tail region, lateral view. Scale bars: A = 200 µm, B = 1 mm

aer 1842). No recent documentation has been made for orb-web genus *Caerostris*, not even for the whole family of Araneidae in last few decades (Penney & Bennet, 2006).

It must be noted that most of the spider-mermithid interactions come from the Palaearctic and Nearctic region in mild climate of Europe and Northern America (e.g. Penney & Bennet, 2006; Meyer, 2014). Records from sub-tropical and/or tropical bioregions (especially in Asia) are very scarce and usually only with individual findings (Rodrigues *et al*., 2005; Zamani, 2014; Ranade & Prakash, 2016; see Fig. 1). We suppose this could be explained by long-term and constant arachnological research in countries of Europe and/or North America. Furthermore, pitfall trapping is among the most frequent collecting methods for spiders in these regions (Schmidt *et al*., 2005). This method, usually used with different preservation mediums, efficiently catches high abundances of various groups of epigeic and other actively moving spiders and thus likely enhances the overall chance of catching a mermithid infected spider (see above and Table 1.). On the other hand, arachnological research in Southeast Asian countries has been neglected and very limited, becoming more progressive only in the last 10 years (e.g. Jaeger & Praxaysombath, 2009, 2011; Wongprom & Košulič, 2016), therefore we suppose it is the main reason for the lack of records on spider-mermithid interactions from southeast Asia (Fig. 1). This hypothesis is also supported by the increased number of reports on parasitized insects as paratenic hosts for spiders throughout various countries of Southeast Asia during the last couple of decades; therefore this suggests wide distribution of mermithids in insect hosts (e.g. Poinar & Chang, 1985; Vythilingam *et al*., 2005; Jitklang *et al*., 2012).

In conclusion our finding is the first record of a mermithid nematode in the host species of orb-web spiders from the genus *Caerostris,* and it represents the first evidence of spider-mermithid interactions from Southeast Asia. However, it is very possible that mermithid parasitism is actually widespread in all Asian countries, since a great variety of spiders have been noted as mermithid hosts from other regions (e.g. Poinar, 1985, 1987, 1990; Penney & Bennett, 2006; Meyer, 2014) and since findings of mermithids in insect paratenic hosts are common throughout southeast Asia (e.g. Jitklang *et al*., 2012). Unfortunately, the following problems: the low chance of detection in nature, insufficient knowledge of this group, the need for adult stages (especially of males) for identification, and the difficulties in rearing mermithids to the adult stage, all complicate the study of mermithid parasitoids of spiders and their biodiversity in South East Asia and elsewhere.

Conflict of interest

Authors state no conflict of interest.

Acknowledgments

We would like to express our thanks to Chaowalit Songsanchote (Kasetsart University) for the finding of the infected spider and for all of his great kindness and friendship during our stay in Thailand. Further, we would like to thank to Anna Šestáková (Western Slovakia Museum) for confirmation of host species identification. We also thank to anonymous reviewer for his comments and corrections that improved the manuscript. The research was supported in part by European Social Fund and the state budget of the Czech Republic under the project Indicators of Trees Vitality (Reg. No. CZ.1.07/2.3.00/20.0265).

References

Ahtiainen, J.J., Alatalo R.V., Kortet, R., Rantala, M.J. (2004): Sexual advertisement and immunen function in arachnid species (Lycosidae). *Behav. Ecol.*, 15: 602–606. DOI: 10.1093/beheco/arh062

Allard, C., Robertson, M.W. (2003): Nematode and dipteran endoparasites of the wolf spider *Pardosa milvina* (Araneae, Lycosidae). *J. Arachnol.*, 31: 139–141. DOI: 10.1636/0161-8202(2003)031

Finch, O.D. (2005): The parasitoid complex and parasitoid-induced mortality of spiders (Araneae) in a Central European woodland. *J. Natur. Hist.*, 39: 2339 – 2354. DOI: 10.1080/00222930502005720

Holm, Å. (1941): Über Gynandromorphismus und Intersexualität bei den Spinnen [Notes on gynandromorphism and intersexuality in spiders]. *Zool. Bidr. Uppsala*, 20: 397 – 414

Iida, H., Hasegawa, H. (2003): First record of a mermithid nematode emerging from the wolf spider *Pardosa pseudoannulata* (Araneae: Lycosidae). *Acta Arachnol.*, 52: 77 – 78. DOI: 10.2476/asjaa.52.77

Jäger, P., Praxaysombath, B. (2009): Spiders of Laos: New species and new records (Arachnida: Araneae). *Acta Arachnol.*, 58: 27 – 51. DOI: 10.2476/asjaa.58.27.

Jäger, P., Praxaysombath, B. (2011): Spiders from Laos with forty-three new records and first results from the province Bolikhamsay and Champasak (Arachnidae: Araneae). *Acta Arachnol.*, 60: 9 – 31. 10.2476/asjaa.60.9.

Jitklang, S., Ahantarig, A., Kuvangkadilok, C., Baimai, V., Adler, P.H. (2012): Parasites of larval black flies (Diptera: Simuliidae) in Thailand. *Songklanakarin J. Sci. Technol.*, 34: 6. DOI: 10.2307/3227000

Leech, R.E. (1966): The spiders (Araneida) of Hazen Camp. *Quaest. Entomol.*, 2: 153 – 212.

Matsuda, M. (1999) Two intersexual spiders of the family Lycosidae from Japan. *Bull. Higashi Taisetsu Mus. Natur. Hist.*, 21: 5 – 54 (In Japanese)

Marc, P., Canard, A., Ysnel, F. (1999): Spiders (Araneae) useful for pest limitation and bioindication. *Agric. Ecosyst. Environ.*, 74: 229 – 273. DOI: 10.1016/S0167-8809(99)00038-9

Meyer, M. (2014): New record of a parasitoid worm (Mermithidae, Nematoda) in a spider of the genus *Trochosa* (Lycosidae). *Arachnol. Mitt.*, 48: 13 – 15. DOI: 10.5431/aramit4803

Michalko, R., Pekár, S. (2015): The biocontrol potential of *Philodromus* (Araneae, Philodromidae) spiders for the suppression of pome fruit orchard pests. *Biol. Control*, 82: 13 – 20. DOI: 10.1016/j.*biocontrol*.2014.12.001

Michalko, R., Pekár, S., Entling, M.H. (2019): An updated perspective on spiders as generalist predators in biological control. *Oecologia*, 189: 21 – 36. DOI: 10.1007/s00442-018-4313-1
Murphy, P.G., Lugo, A.E. (1986): Ecology of tropical dry forest. *Annu. Rev. Entomol.*, 17: 67 – 88. DOI: 10.1146/annurev.es.17.110186.000435
Murphy, F., Murphy, J. (2000): *An introduction to the spiders of South East Asia*. Malaysia: Malaysian Nature Society, 624 pp.
Nickle, W.R. (1972): A Contribution to our Knowledge of the Mermithidae (Nematoda). *J. Nematol.*, 4: 113 – 146
Nikdel, M., Kaiser, H., Niknam, G. (2011). First record Of *Hexamermis cf . albicans* (Siebold, 1848) (Nematoda: Mermithidae) infecting Lepidopteran larvae from Iran. *Nematol. Mediterr.*, 39: 81 – 83
Okochi, T. (1969) Reports on parasites of spiders. *Kishidaia*, 9: 2 – 5 (In Japanese)
Penney, D., Bennett, S.P. (2006): First Unequivocal Mermithid – Linyphiid (Araneae) Parasite – Host Association. *J. Arachnol.*, 34: 273 – 278. DOI: 10.1636/S04-92.1
Pizzi, R. (2009): Parasites of tarantulas (Theraphosidae). *J. Exot. Pet Med.*, 18: 283 – 288. DOI: 10.1053/j.jepm.2009.09.006
Poinar, G.O., Welch, H.E. (1981) Parasites of invertebrates in the terrestrial environment. In W. Slusarski (Ed) *Review of Advances in Parasitology*. Warsaw: Polish Scientific Publications, pp. 947 – 954
Poinar, G.O. (1983): *The natural history of Nematodes*. Englewood Cliffs: Prentice Hall
Poinar, G.O. (1985): Mermithid (Nematoda) parasites of spiders and harvestmen. *J. Arachnol.*, 13: 121 – 128
Poinar, G.O., Chang, P.M. (1985): *Hexamermis cathetospiculae* n.sp. (Mermithidae, Nematoda), a parasite of the rice stemborer, Tryporyza incertulas (Wlk.) (Pyralidae; Lepidoptera) in Malaysia. *J. Nematol.*, 17: 360 – 363
Poinar, G.O., Benton, C.L.B. (1986): *Aranimermis aptispicula* n.g., n.sp. (Mermithidae: Nematoda), a parasite of spiders. *Syst. Parasitol.*, 8: 33 – 38. DOI: 10.1007/BF00010307
Poinar, G.O. (1987): Nematode Parasites of Spiders. In *Ecophysiology of Spiders*. pp. 299 – 308
Poinar, G.O., Early, J.W. (1990): *Aranimermis giganteus* n. sp. (Mermithidae: Nematoda), a parasite of New Zealand mygalomorph spiders (Araneae: Arachnida). *Rev. Nematol.*, 13: 403 – 410
Poinar, G.O. (2000): Heydenius araneus n.sp (Nematoda: Mermithidae), a parasite of a fossil spider, with an examination of helminths from extant spiders (Arachnida: Araneae). *Invertebr. Biol.,* 119, 388 – 393. DOI: 10.1111/j.1744-7410.2000.tb00108.x
Poinar, G.O. (2012): New fossil nematodes in Dominican and Baltic amber. *Nematology* 14, 483 – 488. DOI: 10.1163/156854111X612199
Ranade, S., Prakash, V. (2016): Parasitization of a huntsman spider (Arachnida: Araneae: Sparassidae: *Heteropoda venatoria*) by a mermithid nematode (Nematoda: Mermithidae). *J. Threat. Taxa*, 8: 9595 – 9596. DOI: 10.11609/jott.3105.8.13.9595-9596
Rodrigues, T., Álvares, É.S.S., Machado, É.O., Maria, M. (2005): New records of the family Mermithidae (Nematoda) as parasitoids of spiders (Arachnida: Araneae) in Brazil and Peru. *Rev. Iber. Aracnol.*, 12: 119 – 120
Schmidt, M.H., Clough, Y., Schulz, W., Westphalen, W., Tscharntke, T. (2006): Capture efficiency and preservation attributes of different fluids in pitfall traps. *J. Arachnol.*, 34: 159 – 169. DOI: 10.1636/T04-95.1
Takasuka, K., Korenko, S., Kysilková, K., Štefánik, M., Černecká, L., Mihál, I., Dolejš, P., Holý, K. (2017): Host utilization of koinobiont spider-ectoparasitoids (Ichneumonidae, Ephialtini, Polysphincta genus-group) associated with *Cyclosa* spp. (Araneae, Araneidae) across the Palaearctic. *Zool. Anz.*, 267: 8 – 14. DOI: 10.1016/j.jcz.2017.01.001
Vythilingam, I., Sidavong, B., Chan, S.T., Phonemixay, T., Phompida, S., Krishnasamy, M. (2005): First report of mermithid parasitism (Nematoda: Mermithidae) in mosquitoes (Diptera: Culicidae) from Lao PDR. *Trop. Biomed.*, 22: 77 – 79. DOI: 10.1186/1756-3305-5-131
Wongprom, P., Košulič, O. (2016): First data on spiders (Arachnida: Araneae) from dry dipterocarp forests of Thailand. *Check List*, 12: 1 – 13. DOI: 10.1007/s10841-007-9116-3
Zamani, A. (2014) First report of a mermithid nematode (Enoplea: Mermithida) parasitizing the crab spider *Heriaeus spinipalpus* (Araneae: Thomisidae). *Acta Arachnol.*, 63: 63 – 64. DOI: 10.2476/asjaa.63.63

DOI 10.2478/helm-2019-0011

HELMINTHOLOGIA, 56, 2: 168 – 174, 2019

Infection patterns of helminths in *Norops brasiliensis* (Squamata, Dactyloidae) from a humid forest, Northeastern Brazil and their relation with body mass, sex, host size, and season

D. M. AMORIM[1]*, R. W. ÁVILA[1, 2]

[1]Programa de Pós-Graduação em Ecologia e Recursos Naturais, Universidade Federal do Ceará. Av. Humberto Monte, Pici, 60455-970, Bloco 902, Fortaleza, CE, Brasil, *E-mail: *darciamorim@hotmail.com*; [2]Departamento de Química Biológica, Universidade Regional do Cariri – URCA, R. Cel. Antônio Luiz, 1161, Pimenta, CEP 63105-000, Crato, CE, Brasil

Article info

Received October 26, 2018
Accepted March 5, 2019

Summary

Climatic and ecological factors can influence the parasite load of a host. Variation in rainfall, body size, and sex of the hosts may be related to the abundance of parasites. This study investigated the helminth fauna associated with a population of *Norops brasiliensis*, together with the effect of host biology (sex, body size, and mass) and variation in rainfall regime on the abundance of helminths. Species of three groups of endoparasites were found (Nematoda, Cestoda, and Trematoda), with nematodes as the most representative taxa with eight species, prevalence of 63.2 %, mean intensity of 4.0 ± 0.58 (1 – 25), and mean abundance of 2.66 ± 0.44 (0 – 25). Nine helminth species are new host records for *N. brasiliensis*. The nematode *Rhabdias* sp. had the highest prevalence (53.3 %). There was no significant relationship between abundance of the trematode *Mesocoelium monas* and host sex or season, although the abundance of this parasite increased significantly with host body size and mass, while abundance of nematodes was related to season and host mass. This study increases the knowledge about the diversity of helminth fauna associated with *N. brasiliensis*, revealing infection levels of hosts from northeastern Brazil.

Keywords: Parasite abundance; Nematoda; Cestoda; Trematoda; Lizards; South America

Introduction

Parasites live in constant interaction with their hosts and can influence their way of life and their relationships with the environment, while using them as a source of food and shelter (Aho, 1990; Lozano, 1991; Thomas *et al*., 2010; Hafer & Milinski, 2016). Knowledge of the helminth fauna of a species can provide crucial information for understanding its behavior, diet, and habitat use (Lozano, 1991; Pereira *et al*., 2012, Cabrera-Guzmán & Garrido-Olvera, 2014; Maia-Carneiro *et al*., 2017).

Climatic factors, such as variation in rainfall and temperature, as well as variation in host biology and parasite life cycle, may influence the community structure of helminths (Aho, 1990; Brito *et al.,* 2014; Araújo-Filho *et al.,* 2016; Maia-Carneiro *et al*., 2017). Host size, mass, sex, and reproductive period can also influence parasitism (George-Nascimento *et al*., 2004; Poulin & George-Nascimento, 2007; Pereira *et al*., 2012; Araújo-Filho *et al*., 2016; Oliveira *et al*., 2017).

Lizards act as hosts for many groups of parasites, are good models for ecological studies because they are easy to identify, and are widely distributed around the world (Huey & Pianka, 1981; Ávila & Silva, 2010; Roca & Galdón, 2010). In Brazil, the number of studies on the helminth fauna of lizards is increasing (Ávila & Silva, 2010; Ávila *et al*., 2011; Pereira *et al*., 2012; Macedo *et al*., 2017), although approximately 70 % of the lizard species from the Northeast have still not been investigated for parasites (Ávila *et al.,* 2012; Ribeiro *et al*., 2012; Araújo-Filho *et al*., 2016; Cabral *et al*., 2018).

* – corresponding author

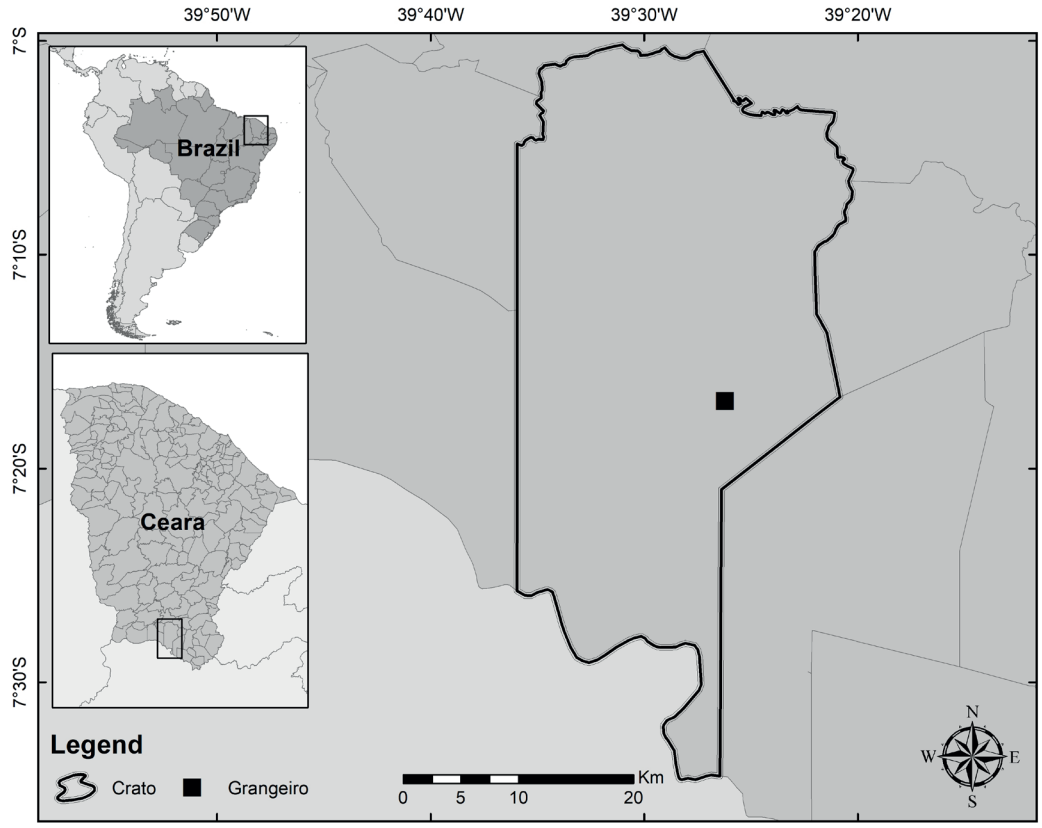

Fig. 1 Location of the studied population of *Norops brasiliensis* in an area of humid forest in Grangeiro, municipality of Crato, state of Ceará, Northeast Brazil.

Norops brasiliensis (Vanzolini & Williams, 1970), is considered endemic to the Cerrado domain, but can also be found in open Amazonian formations (Ávila-Pires 1995; Mesquita *et al.,* 2015). Thus far, for the state of Ceará, *N. brasiliensis* was recorded only in Chapada do Araripe, an area of Cerrado and humid forest (Ribeiro *et al.,* 2012; Roberto & Loebmann, 2016). *Norops brasiliensis* appears to have a generalist diet, feeding mainly on insects, spiders, and gastropods (Mesquita *et al.,* 2015).

Ávila *et al.,* (2011) reported the occurrence of helminths in *N. brasiliensis*, but were limited to a small sample from areas of Cerrado, while Ribeiro *et al.,* (2012) reported parasite infection in the respiratory tract of hosts from areas of humid forest in Northeast Brazil. The present study reports the helminth fauna associated with a population of *N. brasiliensis*, and the effect of host biology (sex, body size, and mass) and variation in rainfall regime on helminth abundance in an area of humid forest in Northeast Brazil.

Material and Methods

This study was carried out on the hillside slope of Chapada do Araripe in an area of humid forest (Tropical Sub-perennial Cloud Forest), at a point located on the Trilha Ecológica do Clube Grangeiro (07°16' S, 39°26' W), the study area is located in Área de Proteção Ambiental do Araripe-APA in the municipality of Crato, state of Ceará, Northeast Brazil (Fig. 1). The area has temperature annual less surrounding areas of Caatinga, ranging from 24 to 26 °C (IPECE 2015).

The rainy season occurs from January to April, while dry season extends from May to December (IPECE 2015). During data collection, the region received a rainfall volume of 100 mm on dry season, and 550 mm on rainy season (Funceme 2016; 2017). Data on rainfall levels were gathered using monthly means from the Fundação Cearense de Meteorologia e Recursos Hídricos – FUNCEME (Foundation of Meteorology and Hydric Resources of Ceará State).

Specimens of *N. brasiliensis* were collected weekly between October 2016 and July 2017. Upon capture specimens were immediately killed with Sodium Thiopental (Thiopentax®), had the snout-vent-length (SVL) taken with digital caliper (precision: 0.01 mm), and were weighed with Pesola® spring scales (precision: 0.1g). The specimens were fixed with 10 % formaldehyde (Franco and Salomão 2002) and vouchers were deposited in the Coleção Herpetológica of the Universidade Regional do Cariri (URCA–H 12667 – 12724, 13004 – 13010, 13012 – 13014; 13016 – 13019; 13093 – 13094; 13129; 13131).

Lizards were dissected and their gastrointenstinal tract, lungs, and body cavity were examined for endoparasites. Cestodes and Trematodes were dehydrated in an increasing series of alcohol,

Table 1. Prevalence (P), mean abundance (MA), and mean intensity of infection (MII) with standard error (SE) and range (R), and infection site (IS) of the helminth community associated with *Norops brasiliensis* (n = 77) in an area of humid forest in Northeast Brazil. Mean values ± standard error. SI – small intestine, ST – stomach, LI – large intestine, C – coelom, L – lung.

Helminth species	P%	MA ± SE (R)	MII ± SE (R)	IS
Nematoda				
Oswaldocruzia bainae	7.8	0.10 ± <0.1 (0–2)	1.33 ± 0.21 (1–2)	SI
Skrjabinellazia sp.	1.3	0.013 ± <0.1 (0–1)	1 ± 0 (1–1)	SI
Parapharygondon sceleratus	18	0.42 ± 0.1 (0–4)	2.3 ± 0.3 (1–4)	SI, LI
Physaloptera retusa	7.8	0.4 ± 0.2 (0–19)	4.8 ± 2.8 (1–19)	ST
Onchocercidae indet.	1.3	0.013 ± <0.1 (0–1)	1 ± 0 (1–1)	ST
Ophidascaris sp.	1.3	0.013 ± <0.1 (0–1)	1 ± 0 (1–1)	ST
Cosmorcercidae indet.	2.6	0.04 ± <0.1 (0–2)	1.5 ± 0.5 (1–2)	LI, C
Rhabdias sp.	**53**	1.67 ± 0.4 (0–25)	3.17 ± (1–25)	L
Trematoda				
Mesocoelium monas	**33**	1.66 ± 0.4 (0–18)	5.12 ± 1.0 (1–18)	SI
Cestoda				
Oochoristica sp.	1.3	0.013 ± <0.1 (0–1)	1 ± 0 (1–1)	LI

stained with the hydrochloric carmine technique, and cleared in eugenol, while nematodes were cleared in lactophenol. The specimens were mounted on temporary slides and analyzed using a light microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany). The samples were later stored in 70 % ethanol and deposited in the Coleção Parasitológica of the Universidade Regional do Cariri (URCA-P). Parasite prevalence, intensity, and abundance were calculated according to Bush et al. (1997).

Generalized linear models (GLM), adopting the Poisson distribution, were used to evaluate if parasite abundance, considering the species of the groups Nematoda and Trematoda separated, was influenced by the period of collection of the host (dry or rainy season), and/or host sex, snout-vent-length, and mass. The R software was used for all statistical analyses (R Development Core Team 2012).

Ethical Approval and/or Informed Consent

The collecting methods were defined and authorized by the regulatory agency in Brazil Instituto Chico Mendes de Conservação da Biodiversidade, who granted permission for the collection of the animals (ICMbio/SISBio 57530-1, and 8383-1) and ethics committee of Universidade Regional do Cariri (CEUA/URCA, process # 00260/2016.1).

Results

A total of 77 adult lizards, including 26 females (SVL mean: 61.35 ± 3.37, range 55.86 – 67.27 mm) and 51 males (SVL mean: 61.47 ± 4.27, range 52.91 – 70.31mm) were examined. Sixty-four specimens were infected by at least one helminth species, with a total

Table 2. Generalized linear model (GLM) values considering abundance of species of the groups Nematode and Trematoda separated in relation to season, sex, snout-vent-length (SVL) and mass for *Norops brasiliensis* in an area of humid forest in Northeast Brazil. Significant p-values are in bold.

	Nematoda			Trematoda		
GLM	**SD**	**z value**	**P**	**SD**	**z value**	**P**
Intercept	1.253828	-0.749	0.454	1.27401	5.906	**< 0.0001**
SVL	0.025743	-0.251	0.802	0.02482	-7.553	**< 0.0001**
Sex	0.16523	1.915	0.0555	0.19405	-0.551	0.582
Mass	0.081017	4.1	**< 0.0001**	0.08467	9.538	**< 0.0001**
Season	0.142172	2.001	**0.0454**	0.18784	0.484	0.629

of 334 helminths specimens collected. Ten species of helminths were recorded associated with *N. brasiliensis* (Table 1), distributed among three groups: Nematoda, with eight taxa, and Trematoda and Cestoda, with a single species each. All taxa, except *Rhabdias* sp., represent new records for *N. brasiliensis.*

The hosts presented a mean richness of 0.9 ± 0.1 for Nematoda species. Overall prevalence was 63.2 %, overall mean abundance was 2.66 ± 0.44 (0 – 25), and overall mean intensity of infection was 4.0 ± 0.58 (1 – 25) for nematodes. For Cestoda and Trematoda see Table 1.

The mean abundance of trematodes was significantly positive related to host SVL (z = -7.553, P < 0.001) and host mass (z = 9.538, P < 0.001). However, the relationship among mean abundance of trematodes and host sex (z = -0. 551, P = 0.582), and season (z = 0.484, P = 0.629) was not significant. Mean abundance of nematodes was significantly positive related to host mass (z = 4.100, P = < 0.001), and season (z = 2.001, P = 0.045), but not to sex (z = 1.915, P = 0.055) and SVL (z = -0.251, P = 0.802) (see Table 2). Cestoda occurred in a single host, with only one parasite recorded.

Discussion

Species of the phylum Nematoda showed high abundance and prevalence of infection in *N. brasiliensis*, which is a common pattern found in many studies with lizards worldwide, including members of the family Dactyloidae (Goldberg *et al*., 2006; Ávila & Silva, 2010; Cabrera-Guzmán & Garrido-Olvera, 2014; Araújo-Filho *et al*., 2016; Sowemimo & Oluwafemi, 2017; Cabral *et al*., 2018). The high abundance of these endoparasites in reptiles may be related to their life cycle, diet, and lifestyle that favors infections by this group (Cabrera-Guzmán & Garrido-Olvera, 2014; Sowemimo & Oluwafemi, 2017).

All helminth species found, with the exception of *Rhabdias* sp. (a new species in process of formal description), represent new records for *N. brasiliensis*. Ávila *et al.,* (2011) reported parasitic infection by the nematode *Subulura lacertilia* in a population of *N. brasiliensis* from a Brazilian Cerrado area, and Ribeiro *et al.,* (2012) reported infection by *Rhabdias* sp. in the respiratory tract of this host species from areas of humid forest in Northeast Brazil. Ribeiro *et al.,* (2012) found prevalence of 28.6 % for *Rhabdias* sp., in *N. brasiliensis* which is lower than what we observed the present study (53.3 %). The high prevalence reported herein may be due to factors such as microhabitat use and host behavior (Aho, 1990; Goldberg *et al.,* 2006). *Rhabdias* (Stiles & Hassall, 1905) are nematodes with monoxenic life cycles, with infection by active penetration of the larva through the skin of the host (Anderson, 2000), commonly found infecting the lungs of lizards (Goldberg *et al.,* 2006; Vrcibradic *et al.,* 2007; Almeida *et al.,* 2009; Ávila *et al.,* 2011, Ribeiro *et al.,* 2012; Cabrera-Guzmán & Garrido-Olvera, 2014; Dorigo *et al.,* 2014). For the genus *Norops* in Brazil, there are reports of species of *Rhabdias* infecting only the species *N. fuscoauratus* (D'Orbigny 1937) and *N. brasiliensis* (Goldberg *et al.,* 2006; Ribeiro *et al.,* 2012).

In the present study, the monoxenic parasites *Parapharygondon sceleratus* and *Oswaldocruzia bainae,* as well as *Rhabdias* sp., showed high prevalence but low mean intensity of infection. This may be related to the evolutionary strategies of the parasites and to the host's ecology (Aho, 1990; Goldberg *et al.,* 2006). This is the first record of *P. sceleratus* infecting a species of *Norops*, while *O. bainae* has been previously recorded infecting two species of the genus in Brazil, *N. fuscoauratus* and *N. chrysolepis* (Ávila & Silva, 2010).

The heteroxenic nematode *Physaloptera retusa* (Rudolphi 1819) was found in this study with high prevalence and mean intensity of infection, which may be related to host diet (Aho, 1990; Anderson, 2000). *Norops brasiliensis* feeds mainly on termites, crickets, gastropods, insect larvae, and spiders (Mesquita *et al.,* 2015), being exposed to infection by the helminths that may be using these invertebrates as intermediate hosts (Aho, 1990; Anderson, 2000; Cabrera-Guzmán & Garrido-Olvera, 2014).

Other nematodes, (Onchocercidae Leiper 1911 (Filarioidea); Cosmocercidae Travassos 1925; *Ophidascaris* sp.; *Skrjabinelazia* sp.) and the cestode *Oochoristica* sp. were also recorded herein with low prevalence and mean infection intensity, which could be explained by the life cycle of the parasites or as casual infections (Aho, 1990; Anderson, 2000). Nonetheless, they are common parasites of the intestines of amphibians and reptiles, including lizards (Ávila & Silva 2013; Campião *et al.,* 2014; Amorim *et al.,* 2017).

The trematode *Mesocoelium monas* (Rudolphi 1819) was also recorded in this study, being the helminth with the second highest prevalence (Table 1). This parasite species generally uses gastropods as intermediate hosts (Escudero & Murillo, 2007). *Mesocoelium monas* is a widely distributed parasite, and has been previously documented infecting lizards of the genus *Norops* (Ávila & Silva, 2010). The high prevalence recorded herein may be related to the diet and habitat use of the hosts (Escudero & Murillo, 2007; Mesquita *et al.,* 2015).

The abundance of species of nematodes in *N. brasiliensis* was significantly different regarding rainfall regime. However, the abundance of *M. monas* did not differ significantly. In some species, parasite infection may be related to seasonal variation, due to the life cycle of the parasites and possible changes in habits of the hosts throughout the year (Aho, 1990; Brito *et al.,* 2014; Araujo-Filho *et al.,* 2016). *Norops brasiliensis* may be exposed to *M. monas* throughout the year, because they prefer humid microhabitats and are active in the coldest hours of the day, a lifestyle common to the intermediate hosts of these parasites, which may explain their abundance being not related to season (Escudero & Murillo 2007; Vitt *et al.,* 2008; Mesquita *et al.,* 2015).

There were no significant differences in the average abundance of parasites regarding host sex. Previous studies also reported no occurrence of intersexual variation related to the abundance of parasites (Amo *et al.,* 2005; Sowemimo & Oluwafemi, 2017;

Cabral *et al.,* 2018; Ribeiro *et al.,* 2018). This may be related to the reproductive period, which may indicate that investment in reproduction is greater than in defense against parasites for both sexes (Zuk & McKean, 1996; Amo *et al.,* 2005; Roberts *et al.,* 2004; Martin *et al.,* 2008; Cabral *et al.,* 2018), as well as behavior and lifestyle of the host species that influence the exposure to parasites (Sowemimo & Oluwafemi, 2017; Ribeiro *et al.,* 2018).

The abundance of trematodes was significantly related to host body size and mass, while for nematodes the abundance was related only to mass. Generally, larger hosts (body size and/or mass) have greater capacity to provide shelter and resources for the development of a greater number of parasites (Aho, 1990; George-Nascimento *et al.,* 2004; Poulin & George-Nascimento, 2007). Another important factor is the life span of the hosts because, compared to juveniles, older individuals have a greater period of time for exposure to infection by parasites, as well as larger body size and mass, which may result in higher rates of parasitism (Aho, 1990; Poulin, 1997).

The results of the present study showed that the seasonality does not influence the abundance of trematodes, but is significant for infection with nematodes. The sex of the hosts did not influence the parasitic infection of *N. brasiliensis* for any of the parasites groups. However, body size and mass of the host were significantly related to helminth abundance, suggesting that larger individuals with longer exposure time are more susceptible to helminth infections. This study also highlights the apparent variation in relation to infection by species of Nematoda and Trematoda groups, which may be related to the life cycle of endoparasites and/or a host response to exposure to these groups of helminths, although these are issues that still need to be addressed.

This study contributes to the knowledge of the helminthological fauna of *N. brasiliensis* by revealing infection levels, besides reporting nine new parasite records for this host species, increasing the knowledge of helminths of South American lizards.

Acknowledgements

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, for the Master's scholarship granted to DMA, and for a research fellowship to RWA (# 303622/2015-6), and WO Almeida for permission to use the microscope with an image analyzer for the identification of helminths.

Conflict of Interest

Authors state no conflict of interest.

References

Aho, J.M. (1990): Helminth communities of amphibians and reptiles: comparative approaches to understanding pattern and process. In: Esch GW, Bush AO, Aho JM (eds) *Parasite communities: patterns and processes*. Chapman and Hall, London, pp 157 – 195

Amo, L. Fargallo, J.A., Martinez-Padilla J., Millán J., López, P., Martín, J. (2005): Prevalence and intensity of blood and intestinal parasites in a field population of a Mediterranean lizard, *Lacerta lepida*. *Parasitol. Res*., 96(6): 413-417. DOI: 10.1007/s00436-005-1355-1

Almeida, W.O., Ribeiro, S.C., Santana, G.G., Vieira, W.L.S., Anjos., L.A., Sales, D.L. (2009): Lung infection rates in two sympatric Tropiduridae lizard species by pentastomids and nematodes in northeastern Brazil. *Braz. J. Biol*., 69: 963 – 967. DOI: 10.1590/S1519-69842009000400027

Amorim, D.M., Silva, L.A.F., Morais, D.H., Silva, R.J., Ávila, R.W. (2017): *Aplectana nordestina* n. sp. (Nematoda: Cosmocercidae) parasitizing *Leposternon polystegum* (Squamata: Amphisbaenidae) from Northeastern, Brazil. *Zootaxa*., 4247: 83 – 88. DOI: 10.11646/zootaxa.4247.1.12

Anderson, R.C. (2000): *Nematode Parasites of Vertebrates, their Development and Transmission*. 2nd Edition, Walingford, UK, CABI Publishing, 650 pp.

Araújo-Filho, J.A., Brito, S.V., Lima, V.F., Pereira, A.M.A., Mesquita, D.O., Albuquerque, R.L., Almeida, W.O. (2016): Influence of temporal variation and host condition on helminth abundance in the lizard *Tropidurus hispidus* from north-eastern Brazil. *J. Helminthol*., 4:1 – 8. DOI: 10.1017/S0022149X16000225

Ávila, RW, Cardoso, M.W., Oda, F.H., Silva, R.J. (2011): Helminths from lizards (Reptilia: Squamata) at the Cerrado of Goiás state, Brazil. *Comp. Parasitol*., 78(1):120 – 128. DOI: 10.1654/4472.1

Ávila, R.W., Silva, R.J. (2013): Helminths of lizards from the municipality of Aripuanã in the southern Amazon region of Brazil. *J. Helminthol*., 87(01):12 – 16. DOI: 10.1017/S0022149X11000769

Ávila, R.W., Silva, R.J. (2010): Checklist of helminths from lizards and amphisbaenians (Reptilia, Squamata) of South America. *J. Venom. Anim. Toxins. Incl. Trop*. Dis. 16:1 – 30. DOI: 10.1590/S1678-91992010000400005

Ávila, R.W., Anjos, L.A., Ribeiro, S.C., Morais, D.H., Silva, R.J., Almeida, W.O. (2012): Nematodes of lizards (Reptilia: Squamata) from Caatinga biome, northeastern Brazil. *Comp. Parasitol*., 79(1):56 – 63. DOI: 10.1654/4518.1

Ávila-Pires, T.C.S. (1995): Lizards of Brazilian Amazonia (Reptilia: Squamata). *Zool. Verh*., 299: 1 – 706

Brito, S.V., Ferreira, F.S., Ribeiro, S.C., Anjos, L.A., Almeida, W.O., Mesquita, D.O., Vasconcellos, A. (2014): Spatial-temporal variation of parasites in *Cnemidophorus ocellifer* (Teiidae) and *Tropidurus hispidus* and *Tropidurus semitaeniatus* (Tropiduridae) from Caatinga areas in northeastern Brazil. *Parasitol. Res*., 113:1163 – 1169. DOI: 10.1007/s00436-014-3754-7

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W. (1997): Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol*., 83(4): 575 – 583. DOI: 10.2307/3284227

Bursey, C.R., Goldberg, S.R., Vitt, L.J. (2007): New species of *Rhabdias* (Nematoda: Rhabdiasidae) and other helminths from *Norops capito* (Sauria: Polychrotidae) from Nicaragua. *J. Parasi-*

tol., 93: 129 – 131. DOI: 10.1645/GE-887R.1
Cabral, N.A., Teles, D.A., Brito, S.V., Almeida, W.O., Anjos, L.A., Guarnieri, M.C., Ribeiro, S.C. (2018): Helminth parasites of *Mabuya arajara* Rebouças-Spieker, 1981 (Lacertilia: Mabuyidae) from Chapada do Araripe, northeastern Brazil. *Parasitol. Res*., 117(4), 1185-1193. DOI: 10.1007/s00436-018-5797-7
Cabrera-Guzmán, E., Garrido-Olvera, L. (2014): Helminth parasites of the lesser scaly anole, *Anolis uniformis* (Squamata: Dactyloidae), from Los Tuxtlas, Southern Mexico: evidence of diet and habitat use. *S. Am. J. Herpetol*., 9: 183 – 189. DOI: 10.2994/SAJH-D-14-00035.1
Campião, K.M., Morais, D.H., Dias, O.T., Aguiar, A., Toledo, G., Tavares, L.E.R., Silva, R.J. (2014): Checklist of helminth parasites of amphibians from South America. *Zootaxa* 3843: 1 – 93. DOI: 10.11646/zootaxa.3843.1.1
Dorigo, T. A., Maia-Carneiro, T., Almeida-Gomes, M., Siqueira, C.C., Vrcibradic, D., Van Sluys, M., Rocha, C.F.D. (2014): Diet and helminths of *Enyalius brasiliensis* (Lacertilia, Iguania, Leiosauridae) in an Atlantic Rainforest remnant in southeastern Brazil. Brazil. *Braz. J. Biol*., 74: 199 – 204. DOI: 10.1590/1519-6984.07612
Escudero, M.B., Murillo, S.A. (2007): Trematodos Digéneos de *Chaunus marinus* (Anura: Bufonidae) en el Municipio de Quibdó, Chocó [Digenetic Trematodes of Chaunus marinus (Anura: Bufonidae) in the Quibdó Municipality, Chocó]. *Rer. Inst Univ Tecnol del Chocó Investig Biodivers y Desarrollo.,* 26: 13 – 17. DOI: 10.18636/biodesarrollo.v26i2.475 (In Spanish)
Franco, L.F., Salomão, M.G. (2002): *Técnicas de coleta e preparação de vertebrados para fins científicos e didáticos* [*Techniques collection and preparation of vertebrates for scientific and educational purposes*]. São Paulo: Instituto Pau Brasil de História Natural. 348 pp. (In Portuguese)
Fundação Cearense de Meteorologia e Recursos Hídricos (2016): Funceme [Foundation of Meteorology and Hydric Resources of Ceará State]. In: Zoneamento Geoambiental do Ceará: Mesorregião do Sul cearense [Geoenvironmental zoning of Ceará state: southern Mesor- region of Ceará]. Retrieved December 26, 2018 from http://www.funceme.br/ Retrieved December 26, 2018 (In Portuguese)
George-Nascimento, M., Muñoz, G., Marquet, P.A., Poulin, R. (2004): Testing the energetic equivalence rule with helminth endoparasites of vertebrates. *Ecology Letters*., 7: 527 – 531. DOI: 10.1111/j.1461-0248.2004.00609.x
Goldberg, S.R., Bursey, C.R., Vitt, L.J. (2006): Parasites of two lizard species, *Anolis punctatus* and *Anolis transversalis* (Squamata: Polychrotidae) from Brazil and Ecuador. *Amphibia–Reptilia*., 27: 575 – 579. DOI: 10.1163/156853806778877068
Hafer, N., Milinski, M. (2016): Inter-and intraspecific conflicts between parasites over host manipulation. *Proc. R. Soc*., B 28: 1 – 9. DOI: /10.1098/rspb.2015.2870
Huey, R.B., Pianka, E.R. (1981): Ecological consequences of foraging mode. *Ecology* 62: 991 – 999. DOI: 10.2307/1936998
Ipece – Instituto de Pesquisa e Estatística Econômica do Ceará [Institute of Research and Economic Strategy of Ceará] (2015): Perfil básico municipal: Crato [Basic profile of the municipality of Crato]. Retrieved November 07, 2017 from http://www.ipece.ce.gov.br/perfi l_basico_municipal/2016/Aiuaba.pdf (In Portuguese)
Lozano, G.A. (1991): Optimal foraging theory: a possible role for parasites. *Oikos*., 60: 391 – 395. DOI: 10.1111/ecog.01720
Macedo, L.C., Gardner, S.L., Melo, F.T.V., Giese, E.G., Santos, J.N. (2017): Nematodes Parasites of Teiid Lizards from the Brazilian Amazon Rainforest. *J. Parasitol*., 103(2), 176 – 182. DOI: 10.1645/16-69
Maia-Carneiro, T., Motta-Tavares, T., Ávila, R.W., Rocha, C.F. (2017): Helminth infections in a pair of sympatric congeneric lizard species. *Parasitol. Res.,* 1 – 8. DOI: 10.1007/s00436-017-5672-y
Martin, L.B., Weil, Z.M., Nelson, R.J. (2008): Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. *Philos. T. R. Soc. B*., 363(1490): 321 – 339. DOI: 10.1098/rstb.2007.2142
Mesquita, D.O., Costa, G.C., Figueredo, A.S., França, F.G., Garda, A.A., Bello Soares, A.H., Werneck, F.P. (2015): The autecology of *Anolis brasiliensis* (Squamata, Dactyloidae) in a Neotropical Savanna*. Herpetol. J*., 25: 233 – 244
Oliveira, B.H.S., Teixeira, A.A.M., Queiroz, R.N.M., Araujo-Filho, J.A., Teles, D.A., Brito, S.V., Mesquita, D.O. (2017): Nematodes infecting *Anotosaura vanzolinia* (Squamata: Gymnophthalmidae) from Caatinga, northeastern Brazil. *Acta Herpetol*., 12: 103 – 108. DOI: 10.13128/Acta_Herpetol-18765
Pereira, F.B., Gomides, S.C., Sousa, B.M., De Souza Lima, S., Luque, J.L. (2012): The relationship between nematode infections and ontogeny and diet of the lizard *Tropidurus torquatus* (Wied, 1820) (Squamata: Tropiduridae) from the Atlantic Rainforest in south-eastern Brazil. *J. Helminthol*., 87: 364 – 370. DOI: 10.1017/S0022149X12000466
Poulin, R., George-Nascimento, M. (2007): The scaling of total parasite biomass with host body mass. Int. *J. Parasitol*., 37: 359 – 364. DOI: 10.1016/j.ijpara.2006.11.009
R Development Core Team R. **(**2012): A language and environment for statistical computing: R Foundation for Statistical Computing version 2.15.1 [computer software]. Austria. URL http://nbcgib.uesc.br/mirrors/cran/
Ribeiro, S.C., Ferreira, F.S., Brito, S.V., Teles, D.A., Ávila, R.W., Almeida, W.O., Anjos, L.A., Guarnieri, M.C. (2012): Pulmonary infection in two sympatric lizards, *Mabuya arajara* (Scincidae) and *Anolis brasiliensis* (Polychrotidae) from a cloud forest in Chapada do Araripe, Ceará, northeastern Brazil. *Braz. J. Biol*., 72(4):929 – 933. DOI: 10.1590/S1519-69842012000500021
Roberts, M.L., Buchanan, K.L., Evans, M.R. (2004): Testing the immunocompetence handicap hypothesis: a review of the evidence. *Anim. Behav*., 68(2):227 – 239. DOI: 10.1016/j.anbehav.2004.05.001
Roberto, I.J., Loebmann, D. **(**2016): Composition, distribution patterns, and conservation priority areas for the herpetofauna of the state of Ceará, northeastern Brazil. *Salamandra*., 52: 134 – 152.

Roca, V., Galdón, M.A. (2010): Haemogregarine blood parasites in the lizards *Podarcis bocagei* (Seoane) and *P. carbonelli* (Pérez-Mellado) (Sauria: Lacertidae) from NW Portugal. *Syst. Parasitol.*, 75: 75 – 79. DOI: 10.1007/s11230-009-9206-6

Sowemimo, O.A., Oluwafemi, T.A. (2017): A Survey of Helminth Parasites of the Lizard, *Agama agama* in Ile–Ife and Ibadan Southwest Nigeria. *J. Bacteriol. Parasitol.*, 8: 1 – 6

Thomas, F., Poulin, R., Brodeur, J. (2010): Host manipulation by parasites: a multidimensional phenomenon. *Oikos*., 119: 1217 – 1223. DOI: 10.1111/j.1600-0706.2009.18077.x

Vanzolini, P.E., Williams, E.E. (1970): South American anoles: the geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria, Iguanidae). *Arq. Zool.*, 19: 1 – 298

Vitt, L.J., Shepard, D.B., Vieira, G.H., Caldwell, J.P., Colli, G.R., Mesquita, D.O. (2008): Ecology of *Anolis nitens brasiliensis* in Cerrado woodlands of Cantao. *Copeia.,* 2008: 144 – 153. DOI: 10.1643/CP-06-251

Vrcibradic, D., Vicente, J.J., Bursey, C.R. (2007): Helminths infecting the lizard *Enyalius bilineatus* (Iguanidae; Leiosaurinae) from an Atlantic Rainforest area in Espírito Santo state, southeastern Brazil. *Amphib-Reptil* 28: 166 – 169. DOI: 10.1163/156853807779799009

Zuk, M., Mckean, K.A. (1996): Sex differences in parasite infection: patterns and processes. *Int. J. Parasitol.*, 26: 1009 – 1024

sciendo

DOI 10.2478/helm-2019-0004

HELMINTHOLOGIA, 56, 2: 175 – 182, 2019

Research Note

The genus *Rhytidodoides* Price, 1939 (Digenea: Rhytidodidae) in Brazil: New geographic occurrence and report of pathology in the gallbladder

M. R. WERNECK[1]*, A. MASTRANGELLI[2], R. VELLOSO[2], P. BALDASSIN[2], H. JERDY[3], E. C. Q. CARVALHO[3]

[1]*BW Veterinary Consulting. Estrada RJ 102, Km 12, Praia Seca, Araruama, RJ Zip code (CEP) 28970-000, Brazil, E-mail: *maxrwerneck@gmail.com*; [2]CTA Enviromental services. Adrress: Rua Saturnino Rangel Mauro 283, Pontal de Camburi, Vitória, ES, CEP 29062-030, Brazil; [3]North Fluminense State University – Darcy Ribeiro (UENF), Campos dos Goytacazes, Zip code (CEP): 28013-602, Rio de Janeiro State, Brazil

Article info

Received December 3, 2018
Accepted January 11, 2019

Summary

The present note describes the occurrence of *Rhytidodoides intestinalis* and *Rhytidodoides similis* (Digenea: Rhytidodidae) in the gallbladder of two juvenile green turtles (*Chelonia mydas* - Testudines, Cheloniidae) found on the coast of Brazil. Both were detected in gallbladder and intestine of green turtles: *Rhytidodoides similis* (United States, Panama, Costa Rica and Brazil) and *R. intestinais* (United States, Panama and Costa Rica). This note is the first report of *R. intestinalis* in Brazil and South-West Atlantic Ocean. Also the histological lesions caused by the parasites in one gallbladder are described.
Keywords: Brazil; *Chelonia mydas*; Digenea; green turtles; *Rhytidodoides intestinalis*; *Rhytidodoides similis*; South-West Atlantic Ocean

Introduction

Parasites of the family Rhytidodidae Odner, 1926, are found in the intestine and gallbladder of sea turtles. Only two genera are currently accepted for the family: *Rhytidodes* Looss, 1901 [*R. gelatinosus* (Rudolphi, 1819) Looss, 1901] and *Rhytidodoides* Price, 1939 (*R. intestinalis* Price, 1939, *R. similis* 1939 and *R. pricei* Mehrotra & Gupta, 1978) (Price, 1939; Mehrotra & Gupta, 1978; Blair, 2005).

In the Neotropical region (Central and South America), *R. gelatinosus* has been reported in the loggerhead turtle (*Caretta caretta*) in Brazil, the green turtle (*Chelonia mydas*) in Panama and Brazil and the hawksbill turtle (*Eretmochelys imbricata*) in Puerto Rico and Cuba. *Rhytidodoides similis* has been reported in the green turtle in Panama and Brazil. *R. intestinalis* has been reported in the green turtle in Panama and Costa Rica (Werneck and Silva, 2016). This note describes the occurrence of *R. similis* and *R. intestinalis* in green turtles found on the coast of Brazil. The injuries attributed to these parasites in the gallbladder of one host are also described.

Material and Methods

In October 2017, a green turtle was found stranded on Rasa Beach (22°43`39.324``S; 41° 58' 40.476"W) in the municipality of Búzios in the State of Rio de Janeiro, Brazil, and was taken to a rehabilitation center. The turtle (83 cm in curvilinear carapace length-CCL and weighing 28.45 kg) was thin (with bones evident in the plastron region) and lethargic. Treatment involved rehydration with saline solution, preventive antibiotic therapy (enrofloxacin) and dietary supplementation through an esophageal tube. The animal died on the eleventh day of rehabilitation and was immediately submitted to necropsy.

The examination revealed atrophy of the pectoral muscles, which had a gelatinous appearance. Moreover, the entire carcass was

* – corresponding author

Table 1. Morphometric data of *Rhytidodoides similis*, Price, 1939 (Digenea: Rhytidodidae) from *Chelonia mydas* (Testudines, Cheloniidae). Measurements in micrometers as Range (mean).

	Price (1939)	Smith et al. (1941)	Caballero (1954)	Werneck et al. (2015)	Present Report
Site	Gall bladder	Gall bladder	Biliary ducts	Small intestine and Gall bladder	Gall bladder
Locality	USA	USA	Panama	Brazil	Brazil
N	2	?	16	2	4
Body length (mm)	1.3 – 3.8	1.2 – 4.4	2.573 – 2.789	3.55 – 3.7	3.93 – 7.8 (5.0)
Body width (mm)	0.51 – 1.4	0.42 – 1.8	0.747 – 0.847	1.54 – 1.6	1.19 – 1.28 (1.2)
Oral sucker length	150 – 320*	140 – 342* (293)	103 – 114	280 – 330	248 – 308 (277)
Oral sucker width			243 – 247	240 – 370	297 – 326 (314)
Pharynx length	74 – 165	80 – 179	125 – 144	160 – 200	153 – 174 (163)
Pharynx width	63 – 160	50 – 170	110	210	132 – 143 (138)
Ventral sucker length	165 – 370	170 – 385 (300)	289 – 312	210 – 220	302 – 359 (335)
Ventral sucker width			266 – 285	210 – 230	277 – 354 (303)
Esophagus length	220 – 720	–	391 – 448	620 – 700	499 – 856 (638)
Esophagus width	–	–	76 – 95	110	103 – 176 (154)
Anterior Testes length	185 – 400*	102 – 420	232 – 249	330 – 440	367 – 488 (411)
Anterior Testes width		250 – 456	216 – 282	350 – 410	321 – 566 (425)
Posterior Testes length	220 – 480	152 – 532*	299 – 332	420 – 530	381 – 564 (473)
Posterior Testes width	135 – 400		199 – 216	390 – 490	309 – 516 (403)
Cirrus sac length	200 – 800	150 – 700	481 – 515	720 – 900	809 – 853 (827)
Cirrus sac width	160 – 430	150 – 456	199 – 216	220 – 450	295 – 345 (320)
Ovary length	90 – 300*	91 – 280 (250)*	129 – 133	200 – 260	137 – 220 (180)
Ovary width			129 – 137	200 – 210	142 – 240 (194)
Mehlis`gland length	–	–	133 – 152	110 – 140	94 – 211 (134)
Mehlis`gland width	–	–	175 – 190	120 – 130	110 – 196 (141)
Eggs length	63 – 70	36	61 – 65	37 – 60 (45)	47 – 59 (54)
Eggs width	37 – 40	72	38 – 42	20 – 40 (29)	22 – 34 (28)
Distance from (mm)					
To intestinal caeca bifurcation to anterior end	–	–	–	–	1.146 – 1.264 (1.22)
To genital aperture to anterior end	–	–	747 – 863	–	0.871 – 1.05 (0.955)
To ovary to ventral sucker	–	–	–	–	0.810 – 0.916 (0.847)

*Diameter

Table 2. Morphometric data of *Rhytidodoides intestinalis* Price, 1939 (Digenea: Rhytidodidae) from *Chelonia mydas* (Testudines, Cheloniidae). Measurements in micrometers as range (mean).

	Price (1939)	Caballero (1954)	Present Report
Site	Gall bladder	Small intestine	Gall bladder
Locality	USA	Panama	Brazil
N	2	1	3
Body length (mm)	2.0 – 2.1	3.12	2.4 – 2.5 (2.4)
Body width	320 – 335	448	268 – 382 (339)
Oral sucker length	110 – 130*	57	117 – 142 (131)
Oral sucker width		190	129 – 159 (148)
Pharynx length	60 – 70	68	55 – 60 (57)
Pharynx width	55 – 70	76	67 – 69 (67)
Ventral sucker length	103 – 115*	118	115 – 140 (124)
Ventral sucker width		125	88 – 126 (105)
Esophagus length	730 – 830	912	887 – 970 (939)
Esophagus width	–	68	39 – 49 (44)
Anterior Testes length	130*	141	123 – 149 (137)
Anterior Testes width		118	109 – 137 (127)
Posterior Testes length	130*	198	107 – 144 (130)
Posterior Testes width		160	102 – 151(132)
Cirrus sac length	110 – 137	190	149 – 196 (174)
Cirrus sac width	105 – 115	148	124 – 151 (133)
Seminal vesicle length	–	114	-
Seminal vesicle width	–	061	-
Ovary length	90 – 110*	114	94 – 104 (100)
Ovary width		122	85 – 144 (114)
Mehlis`gland length	–	152	72 – 89 (80)
Mehlis`gland width	–	84	62 – 126 (84)
Eggs length	60 – 63	57	51 – 63 (55)
Eggs width	45 – 52	34 – 38	27 – 40 (31)
Distance from (mm)			
To intestinal caeca bifurcation to anterior end	–	–	1.08 – 1.11 (1.11)
To genital aperture to anterior end	–	1.461	1.1 (only in one)
To ovary to ventral sucker	–	–	520 (only in one)

*Diameter

Fig. 1. *Rhytidodoides similis* Price 1939 (Digenea: Rhytidodidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines: Cheloniadae) from Brazil. Ventral view.

pale in color. Samples were taken of the spleen, brain, cerebellum, heart, digestive tract, liver, ocular globe, salt gland, testis, pancreas, respiratory tract, kidneys, urinary tract and gallbladder. The samples were fixed in 10% formalin solution, embedding in paraffin, sliced (thickness: 5 µm), stained with hematoxylin and eosin (HE) and analyzed with a light microscope.

The second host was found dead in July 2018 caught in a fishing net near Ilha Grande (23°7'12.549"S; 44° 8' 3.124"W) in the municipality of Angra dos Reis in the State of Rio de Janeiro, Brazil. The turtle had 68.1 cm CCL and weighing 24.6 kg, tissue samples in this host were not histologically analyzed due to the autolytic state.

During the analysis of the gallbladders, seven specimens of the family Rhytidodidae [three specimens of *R. intestinalis* and four specimens of *R. similis* were found in the first host and only eight *R. similis* were found in the second host]. The parasites were fixed in 70% alcohol, stained with hydrochloric carmine and cleared in a eugenol solution. The specimens were photographed and measured (data expressed as minimum-maximum values in µm) under a microscope (Nikon Eclipse 80i, Kurobane Nikon Co., Ltd., Otawara, Tochigi, Japan) with the aid of the NIS-Elements BR software. Analyses of the parasites were authorized by federal licenses for activities with scientific purposes (SISBIO 30600-1 and 9329-1). The helminths were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (*R. similis* = CHIOC 38590; *R. intestinalis* = CHIOC 38589) in the State of Rio de Janeiro, Brazil.

The identification of the parasites was performed using the genus taxonomic key proposed by Blair (2005), the original description by Price (1939) as well as descriptions by Smith et al. (1941), Caballero 1954, Mehrotra & Gupta (1978) and Werneck et al. (2015). Descriptions by Price (1939), Smith et al. (1941), Caballero (1954) and Werneck et al. (2015) were used for the morphometric comparisons of *R. similis* (Table 1) and descriptions by Price (1939), Caballero (1954) and Mehrotra & Gupta (1978) were used for the comparison of *R. intestinalis* (Table 2).

Description of parasites:

Rhytidodoides similis (Figs. 1 and 2; Table 1): body robust, anterior

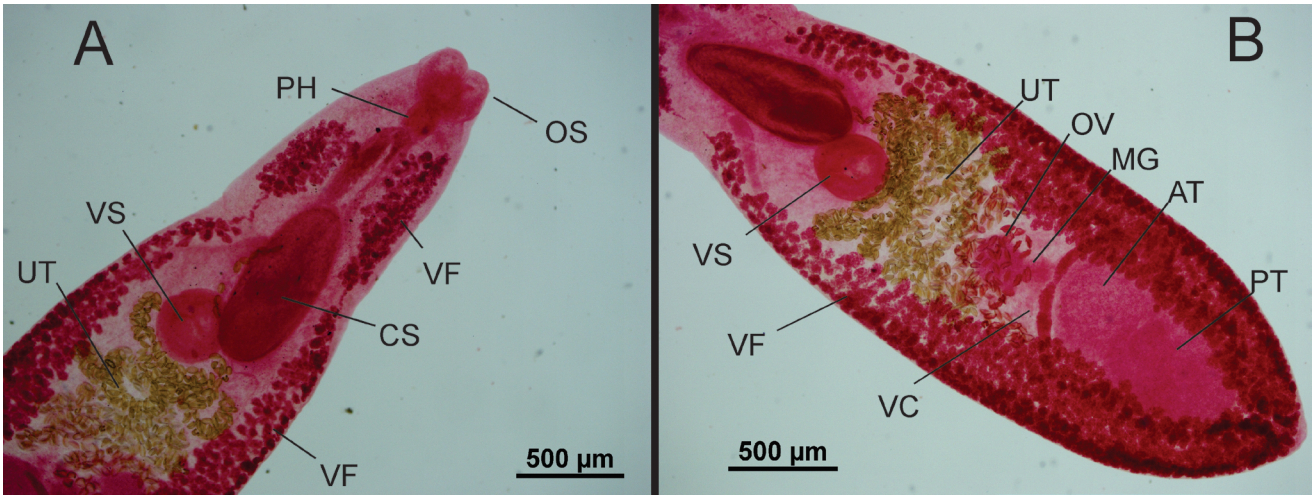

Fig. 2. (A-B) *Rhytidodoides similis* Price 1939 (Digenea: Rhytidodidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines: Cheloniadae) from Brazil. Legend: AT- anterior testes; CS- cirrus sac; MG- Mehlis gland; OS- oral sucker; OV- ovary; PH- pharynx; PT- posterior testis; UT- Uterus; VC- vitelline conduct; VF- vitelline follicles; VS- ventral sucker.

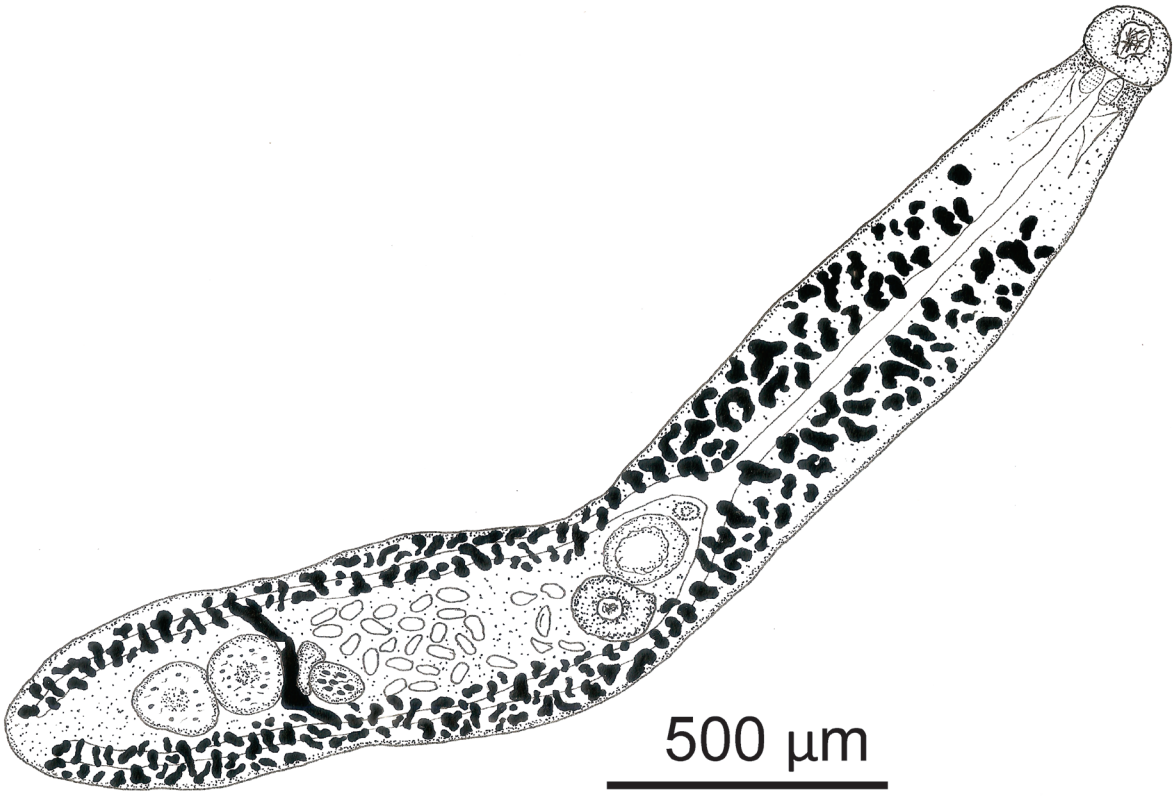

Fig. 3. *Rhytidodoides intestinalis* Price 1939 (Digenea: Rhytidodidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines: Cheloniadae) from Brazil. Ventral view.

tapered, posterior rounded, with constriction in anterior third; terminal oral sucker; pharynx present; acetabulum rounded, posterior to constriction of body; esophagus rectilinear in anterior region of body; two testes in tandem, rounded, located in posterior region of body; cirrus sac voluminous, at level of constriction of body; ovary rounded; Mehlis gland post-ovarian; Vitellaria formed by four groups of follicles, two on each side of the body, two in the anterior region between the pharynx and the anterior portion of the cirrus pouch, these groups connect with the corresponding in the posterior region of the body by a conduit, the second group begin near the region of the half of the cirrus pouch, following in the posterior direction of the body; eggs (18 measured) oval shaped, without polar processes.

Rhytidodoides intestinalis (Figs. 3 and 4; Table 2): body small, thin; oral sucker terminal; pharynx present; esophagus long, rectilinear; testes in tandem, rounded, located in posterior region of body; cirrus sac rounded, near cecal bifurcation; ovary spherical; Vitellaria formed by two groups of follicles, one on each side of the body, from the initial third of the esophagus to the posterior extremity of the body; Mehlis gland posterior to ovary eggs (18 measured) large, elliptical.

Histological description: The histological analysis revealed a giant-cell inflammatory response due to spirorchiids eggs (type 1 and type 3 eggs) in the spleen, brain, cerebellum, heart, large intestine, salt gland and kidneys. In the gallbladder, a mild papillary pattern was found, with multifocal inflammatory infiltrate that reached the submucosal lamina propria and was limited to the basal membrane of the mucosa and bile ducts. The inflammation was composed of lymphocytes, plasmocytes as well as scarce heterophils and eonsinophils and was associated with mildly proliferated (mild fibrosis), reactive, loose connective tissue and moderate hydropic accumulation. The epithelial cells of the mucosa were moderately degenerated, with evident nucleoli and randomly pyknotic nuclei. An amorphous eosinophilic material covered the mucous layer, with cell debris formed by an increase in mucus secretion and necrotic epithelial cells (Fig. 5).

The microscopic diagnosis was moderate, disseminated spirorchidiosis, moderate necrotizing cholecystitis, edema, fibrosis, hydropic degeneration in the gallbladder.

Ethical Approval and/or Informed Consent

For this study formal concern is not required

Remarks

The genus *Rhytidodoides* was created by Price (1939) to group two species: *R. intestinalis* (from the small intestine) and *R. similis* (from the gallbladder). The two species were described based on two specimens collected from a green sea turtle in captivity in the

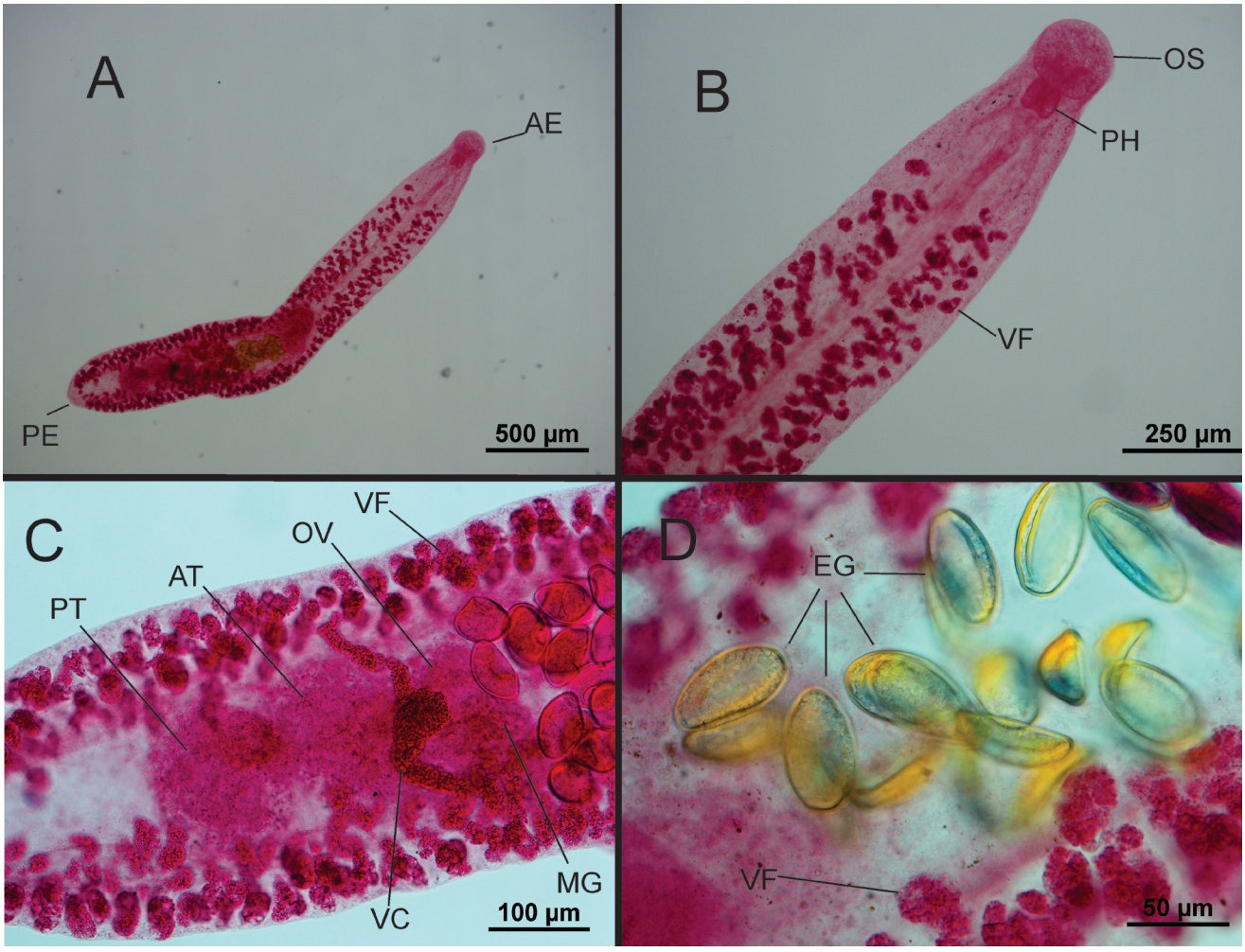

Fig. 4. (A-D) *Rhytidodoides intestinalis* Price 1939 (Digenea: Rhytidodidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines: Cheloniadae) from Brazil. Ventral view. Legend: AE- Anterior end; AT- anterior testes; Eg- eggs; MG- Mehlis gland; OS- oral sucker; OV- ovary; PE- Posterior end; PH- pharynx; PT- posterior testis; VC- vitelline conduct; VF- vitelline follicles.

National Zoological Park, Washington, DC, USA.

Nigrelli (1940) reported the occurrence of *R. similis* collected from the gallbladder of green turtles kept at the New York Aquarium, NY, USA. The author offered few morphometric data (i.e. mean body measuring 2.2 mm in length by 1.97 mm in width among 50 specimens analyzed) and some morphological data: "*In larger specimens the vitellaria are definitely separated into two distinct follicular groupings at level of esophagus and two larger masses extending from level of cecal bifurcation to the extreme posterior end, where they unite*").

Smith et al. (1941) described papillomatous disease in the gallbladder of green turtles associated with the presence of *R. similis*, reporting papillary hyperplasia of the mucosa, fibroplasia, lymphocytic inflammatory infiltrate, dilation of blood and lymphatic vessels, mucous exudate and the presence of trematode eggs enveloped by multinucleated giant cells in the liver. However, the authors did not describe the eggs.

Nigrelli (1941) described the parasitological analysis of 50 green turtles, which revealed 17 species distributed among seven families of trematodes, among which the authors included *R. intestinalis*, *R. similis* and "...a new species of *Rhytidodoides* Price" – all found in the gallbladder. The prevalence of *R. similis* was 65% and papillomatous disease associated with infection by *R. similis* was found in four gallbladders.

Caballero (1954) examined 12 green turtles from Chepillo Island in the Panama Bay and reported the occurrence of nine species of trematodes, including one specimen of *R. intestinalis* collected from the duodenum as well as 17 specimens of *R. similis* collected from the liver. The author described papilloma formation in the bile ducts, leukocytic infiltrate and the proliferation of connective tissue (findings similar to Smith et al. in 1941) as well as the occurrence of inflammatory infiltrate composed of plasmocytes and eosinophils.

Mehrotra & Gupta (1978) described *R. pricei* and listed the differences that established the new species (based mainly on the relationship between the oral sucker and acetabulum). Mehrotra

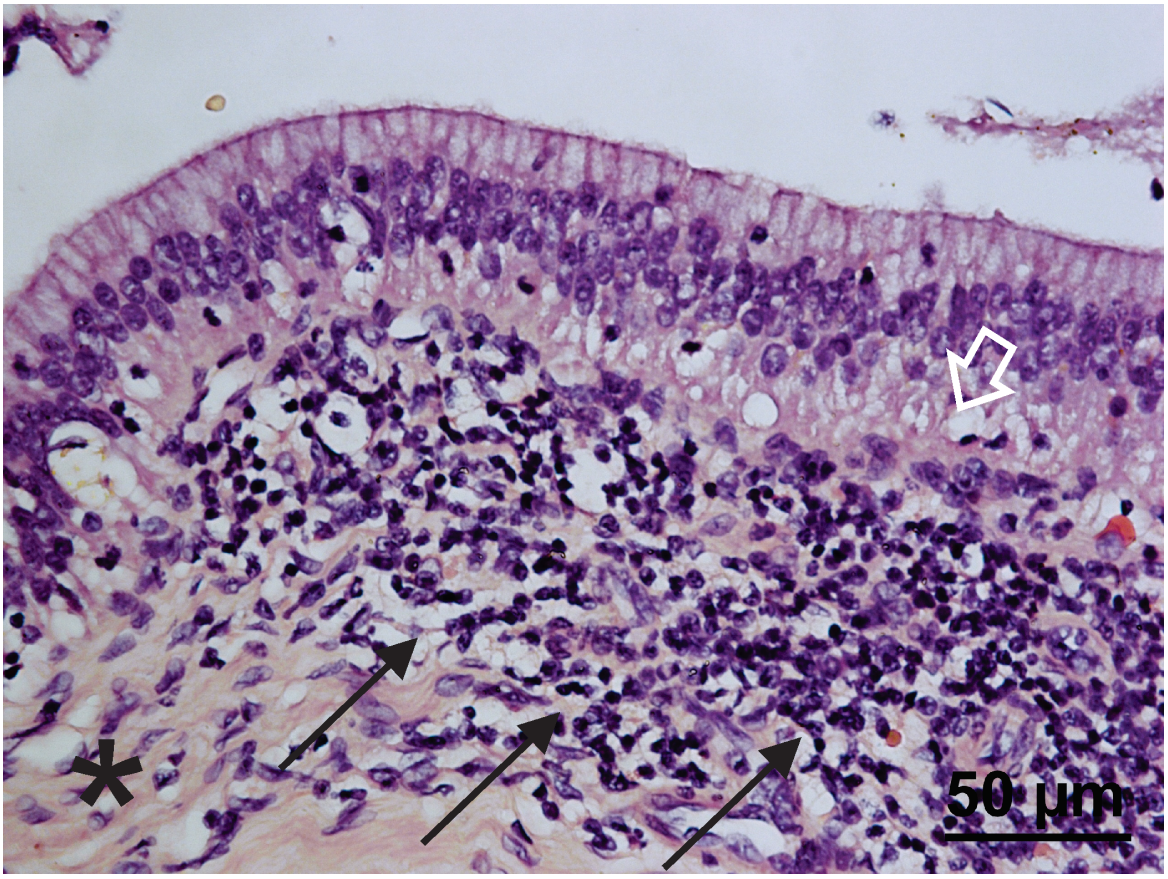

Fig. 5. Histological description of gallbladder: moderate lymphoplasmocytic inflammatory infiltrate, scarce eosinophils and heterophils (black arrows) associated with reactive, proliferated fibrous tissue (*) and epithelial cells from degenerated mucous layer (white arrow).

& Gupta also stated that the specimen found by Caballero (1954) (identified by Caballero as *R. intestinalis*) was actually *R. pricei*. Unfortunately, the authors did not perform a comparative analysis of the specimens collected by Price (1939) and Caballero (1954) and did not state where the parasite had been deposited. Thus *P. pricei* is a synonym of *R. intestinalis* in our opinion.

Santoro et al. (2006) analyzed 40 adult green turtles (all females) in the Tortuguero National Park in Costa Rica and found *R. similis* (in the gallbladder and liver) in 25% of the hosts examined [mean abundance: 2.6 ± 7.1; mean intensity: 10.6 ± 11.3 (range: 1 to 34)] and *R. intestinalis* (in the gallbladder) in 15% of the hosts [mean abundance: 0.2 ± 0.4; mean intensity: 1.2 ± 0.4 (range: 1 to 2)]. Subsequently, Santoro et al. (2007) reported cholycystitis and ectasia of the mucosal glands, with the retention of material and small numbers of leucocytes in one turtle associated with 14 flukes identified as *R. similis*

In an extensive summary, Greiner (2013) described the parasitological analysis of 44 loggerhead sea turtles, 74 green turtles, four hawksbill turtles, two leatherback turtles (*Dermochelys coriacea*), four olive ridley turtles (*Lepidochelys olivacea*) and four Kemp's ridley turtles (*Lepidochelys kempii*) – all from Florida (USA) between 1991 and 2006. The author reported the occurrence of *R. similis* in the liver and gallbladder of 16 green turtles [prevalence: 21.6%; mean intensity: 19.0 (range: 1 to 98)].

More recently, Werneck et al. (2015) described the occurrence of four specimens of *R. similis* in a juvenile green turtle on the coast of Brazil. However, no histological analysis was performed on the gallbladder.

Both *R. similis* and *R. intetinalis* can be considered specialists (see Santoro et al., 2006), as these parasites have only been found in green turtles. *Rhytidodoides similis* has been reported in the United States (Price, 1939; Nigrelli, 1940, 1941; Smith et al., 1941; Greiner 2013), Panama (Caballero, 1954), Costa Rica (Santoro et al., 2006; Santoro et al., 2007) and Brazil (Werneck et al., 2015 and present report). In addition, *R. intestinalis* has been reported in the United States (Price, 1939), Panama (Caballero, 1954), Costa Rica (Santoro et al., 2006) and Brazil (present report).

In the present study, the gallbladder exhibited a mild papillary pattern, multifocal accentuated inflammatory infiltrate that reached the submucosal lamina propria and was limited to the basal membrane of the mucosa. The moderate inflammation composed of lymphocytes, plasmocytes as well as scarce heterophils and eosinophils was associated with mildly proliferated (mild fibrosis), reactive, loose connective tissue and moderate hydropic accu-

mulation. The epithelial cells of the mucosa were moderately degenerated, with evident nucleoli and randomly pyknotic nuclei. An amorphous eosinophilic material covered the mucous layer, with cellular debris formed by an increase in mucus secretion and necrotic epithelial cells (FIG. 5).

The microscopic findings associated with infection by *R. similis* are papillomatous formation, plasmocytic and eosinophilic inflammatory infiltrate, fibroplasia and mucous exudate (Smith et al., 1941; Nigrelli, 1941; Caballero, 1954; Santoro et al., 2006). All these findings are compatible with the results described herein. However, the present report also describes edema in the submucosal lamina propria, the degeneration of epithelial cells of the mucosa, evident nucleoli and mild necrosis of epithelial cells of the mucosa. The edema around the ducts and hydropic degeneration of the mucosa can diminish the lumen and cause biliary stasis. The mild necrosis of the mucosa facilitates infection by infectious agents and makes the bile thicker, which hinders its excretion. Evident nucleoli occurred due to the aggression caused by the inflammation. The morphological findings of the specimens were compatible with data previously described by Price (1939), Caballero (1954) and Blair (2005). The morphometric analysis of the specimens of *R. similis* revealed wider ranges for total body length, acetabulum width, esophagus length and width as well as larger testes and Mehlis glands in comparison to previously published data (Table 1). The analysis of *R. intestinalis* revealed wider ranges regarding the length and width of the oral sucker, acetabulum, anterior testis and cirrus sac as well as larger widths of the ovary and Mehlis gland in comparison to previously published data, whereas the length of the Mehlis gland was smaller compared to data published by other authors (Table 2). Although the morphometrics of both species demonstrated differences for some organs, these differences are believed to be due to the natural variation among individuals and do not compromise the correct identification of the species.

The helminth fauna of green turtle of the coast of Brazil is composed of 36 species of trematodes distributed among ten families and three species of nematodes distributed between two families (Werneck and Silva, 2016, present report).

Conflict of Interest

Authors state no conflict of interest.

Acknowledgments

Biological samples were obtained through the beach monitoring project in the state of Rio de Janeiro, Brazil, which is a requirement established by the federal environmental licensing division of the Brazilian environmental agency (IBAMA), for the exploration of oil and gas by Petrobras at the Santos Basin pre-salt province. In the Espírito Santo State the beach monitoring project is a requirement established by the federal environmental licensing division of the Brazilian environmental agency (IBAMA) process number N°02022.001407/10

References

Blair, D. (2005): Family Rhytidodidae Odner, 1926. In: Jones, A., Bray, R.A., Gibson, D.I. (Eds). *Key to the trematodes*. Vol. 2, London, UK: CABI Publishing, London, pp. 123 – 125

Caballero, y C. E. (1954): Helmintos de la Republica de Panamá. X. Alguns Tremátodos de *Chelone mydas* (L.) tortuga marina comestible Del Océano Pacífico Del Norte [Helminths of the Republic of Panama. X. Some Trematodes of *Chelone mydas* (L.) marine turtle from the North Pacific Ocean.]. *An. Inst. Biol. Univ. Nac. Auton Méx.,* 8: 31 – 58 (In Spanish)

Greiner, E.C. (2013): Parasites of marine turtle. In: Wyneken, J., Lohmann, K.J., Lutz, J.A. (Eds) *The Biology of sea turtles.* Vol. 3, Boca Raton, USA. 475 CRC Press, pp. 427 – 446

Mehrotra, V., Gupta, N.K. (1978): On the status of "*Rhytidodoides intestinalis* Price, 1939" of Caballero 1954 (Trematoda, Rhytidodidae). *Acta Parasitol. Polonica* 25: 207 – 210

Nigrelli, R.F. (1940): Observations on trematodes of the marine turtle, *Chelonia mydas*. *Anat. Rec.* 78: 178

Nigrelli, R.F. (1941): Parasites of the green turtle (*Chelonia mydas* (L.)), with special reference to the rediscovery of trematodes described by Looss from this host species. *J. Parasitol*., 27: 15 – 16

Price, E.W. (1939): A new genus and two new species of digenetic trematodes from a marine turtle. *Proc. Helminthol. Soc. Wash.,* 6: 24 – 25

Smith, G.M., Coates, C.W., Nigrelli, R.F. (1941): A papillomatous disease of the gallbladder associated with infection by flukes, occurring in the marine turtles, *Chelonia mydas* (Linnaeus). Zoologica, 26: 13 – 16

Santoro, M., Greiner, E.C., Morales, J.A., Rodrígues-Ortiz, B. (2006): Digenetic trematode community in nesting green sea turtles (*Chelonia mydas*) from Tortuguero Nacional Park, Costa Rica. *J. Parasitol*., 92: 1202 – 1206. DOI: 10.1645/GE-866R.1

Santoro, M., Morales, J. A., Rodrígues-Ortiz, B. (2007): Spirorchiidiosis (Digenea:Spirorchiidae) and lesions associated with parasites in Caribbean Green turtles (*Chelonia mydas*). *Vet. Rec.,* 161:482 – 486. DOI: 10.1136/vr.161.14.482

Werneck, M.R, Binoti, E, Martins, I.V.F., De Calais Junior, A., Gomes, M.C., Boeloni, J.N., Trazzi, A., Berger, B. (2015): Occurrence of *Rhytidodoides similis* Price, 1939 (Digenea: Rhytidodidae) and lesions due to Spirorchiid eggs in a Green Turtle, *Chelonia mydas* Linnaeus, 1758 (Testudines, Cheloniidae), from Brazil. *Comp. Parasitol.,* 82: 291 – 295. DOI: 10.1654/4747.1

Werneck, M.R., Silva, R.J. (2016): Checklist of sea turtles endohelminths in neotropical region. *Helminthologia*, 53: 211 – 223. DOI: 10.1515/helmin-2016-0045