Parasitology Research (2019) 118:1473–1478
https://doi.org/10.1007/s00436-019-06299-6

HELMINTHOLOGY - ORIGINAL PAPER

Combined morphology and DNA-barcoding to identify *Plagiorhynchus cylindraceus* cystacanths in *Atelerix algirus*

Alejandro Garcia-Salguero[1] · Sofía Delgado-Serra[1] · Jessica Sola[2] · Nieves Negre[2] · Miguel Angel Miranda[1] · Claudia Paredes-Esquivel[1]

Received: 26 October 2018 / Accepted: 18 March 2019 / Published online: 30 March 2019

Abstract

The acanthocephalan parasite *Plagiorhynchus cylindraceus* has a global distribution and utilizes isopods and birds as intermediate and definitive hosts, respectively. Occasionally, mammals of various orders can act as paratenic hosts. In hedgehogs, severe cases have been reported in juvenile specimens due to secondary infections, as a consequence of complete penetrations of the intestinal wall by cystacanths. In a 66-month study period, we found seven larvae of this parasite encysted in both, the peritoneal cavity and intestine of the Algerian hedgehog, *Atelerix algirus* in Majorca. Morphology alone was insufficient to identify the species, due to the lack of previous reports and taxonomy-informative characters. In the present report, we combined the use of morphology and the DNA-barcoding approach to confirm to identify cystacanths as *P. cylindraceus.* This is the first report of this parasite in this hedgehog species. The epidemiological implications will be discussed, including the risk of zoonosis and the importance of using modern approaches to identify immature acanthocephalan larvae in wildlife hosts.

Keywords Cystacanth · DNA-barcoding · Acanthocephala · *Atelerix* · Hedgehogs · Larvae

Introduction

Plagiorhynchus (Prosthorhynchus) cylindraceus (Goeze, 1782) (Acanthocephala: Plagiorhynchidae) is a parasite of cosmopolitan distribution. Its life cycle includes terrestrial isopods and birds, as intermediate and final hosts, respectively. Paratenic hosts have been reported; however, their role in the transmission is not completely understood (Skuballa et al. 2010). Although its final host is usually a bird from the order Passeriformes (Schmidt and Olsen 1964), it has also been reported in other avian orders: Charadriifomes (Amin et al. 1999), Piciformes (Florescu 1984), and Strigiformes (Ferrer et al. 2004). Larval stages of this parasite have been found in the intestine and peritoneal cavity of raccoons (Ching et al. 2000), hedgehogs (Skuballa et al. 2010), shrews (Coady and Nickol 2000) and Australian marsupials (Edmonds 1989). These mammals act as accidental or paratenic hosts (S1). In hedgehogs (Eulipotyphla:Erinaceidae), *P. cylindraceus* has been reported from two species, *Erinaceus europaeus* Linnaeus, 1758 and *Erinaceus roumanicus* Barrett-Halminton, 1900, commonly causing severe damage as a consequence of penetration or attachment to the intestinal wall (Skuballa et al. 2010; Pfaffle et al. 2014).

Atelerix algirus Lereboullet, 1842 (Mammalia: Erinaceidae), the Algerian hedgehog, is a small mammal native to North Africa. It was introduced to the Iberian Peninsula, the Balearic Islands, and the Canary Islands in the thirteenth century (Morales and Rofes 2008). This is the only hedgehog species reported in the Spanish archipelagos. *A. algirus* is not considered vulnerable; however, this species shows a high mortality rate at the wildlife rehabilitation centers, particularly in the Balearic Islands (COFIB, personal communication). Since 2014, seven hedgehog specimens

Alejandro Garcia-Salguero and Sofía Delgado-Serra contributed equally to this study.

Handling Editor: Julia Walochnik

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00436-019-06299-6) contains supplementary material, which is available to authorized users.

✉ Claudia Paredes-Esquivel
claudia.paredes@uib.es

[1] Applied Zoology and Animal Conservation Laboratory, University of the Balearic Islands, Carretera de Valldemossa Km. 7.5, Palma de Mallorca, Spain

[2] Centre de Recuperació de Fauna Silvestre de les Illes Balears (COFIB), Carretera de Sineu Km 15.400, Santa Eugènia, Mallorca, Spain

were found to be infected with an unidentified cystacanth during necropsy. These larvae were found outside the peritoneum or inside the intestine. Since the morphometric approach was not sufficient to determine the species identity at the larval stage, we also used a DNA-barcoding approach. This technique consists of using a short (650 bp) fragment of the *Cytochrome c Oxidase* subunit I (COI), in the mitochondrial genome for sequence comparison and species identification (Hebert et al. 2003b). This parasite was identified as *P. cylindraceus*, and this would be the first report of this acanthocephalan in the Algerian hedgehog. In this article, we discuss the clinical aspects, the epidemiology of the infection, and its potential risks for both wildlife and humans.

Material and methods

This study was conducted in hedgehog specimens (*A. algirus*) that arrived at the Centre per la Recuperació de Fauna Silvestre (COFIB), after rescue from different localities of the Balearic Islands, from January 2014 to June 2018. In this period, a high percentage of animals (36.8–54.9% approximately) died during hospitalization. Those specimens with a total body weight > 350 g were considered adults. After death, most animals were necropsied to investigate the cause of death. Several parasites of unknown identity were found in the intestine peritoneum. Specimens were initially identified as *Moniliformis moniliformis*, since this was the only species of Acanthocephala reported for *A. algirus* (Khaldi et al. 2012).

Specimens were collected and placed in 4% formalin and transported to the Laboratory of Zoology of the University of the Balearic Islands to confirm the morphological identification. Some were preserved in 96% ethanol at − 4 °C for further molecular analysis. Not all the specimens were found in a good state of preservation. Host tissue was removed from individual specimens using forceps. Specimens were clarified with lactophenol and mounted in temporary slides prior observation. Examinations were performed with a light microscope at × 100 and × 200 magnifications. Identifications were carried out using the morphological keys by Amin et al. (1999), and descriptions by Schmidt and Olsen (1964) were reported for this species. Morphological/morphometrical characters measured included the total length of the body and proboscis, the number of rows of hooks, in the proboscis and the number of hooks per row. Since we could not identify specimens at larval stage, these were subjected to a molecular analysis. DNA extraction was carried out using a DNeasy Blood & Tissue Kit (QIAGEN, GmbH, Hilden, Germany), using 30 μl of water instead of 50 μl elution buffer to increase DNA concentration. Total DNA was measured by spectrophotometry using NanoDrop Thermo Scientific (Saveen Werner ApS, Denmark). Only specimens with a DNA extraction higher than 10 ng/μl were included in the analysis. We proceed with the PCR reaction in two viable specimens. Primers used were LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′, and HCO2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3 (Folmer et al. 1994). The 50 μl PCR reaction contained 5 μl of each primer, 1 unit of QIAGEN DNA polymerase 1× reaction buffer (Tris–Cl, KCl), and $(NH4)_2SO_4$, 4 μl dNTPs, 0.15 μl $MgCl_2$, 2 μl of genomic DNA, and PCRs were carried out in a GeneAmp PCR System 2400, Perkin Elmer under the following conditions: an initial denaturation step at 95 °C for 2 min, 35 cycles 92 °C for 30s, 45 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 1 min. PCR products were observed by electrophoresis using a 2% agarose–TBE buffer gel, containing Pronasafe nucleic acid staining solution (Conda laboratories). Samples were purified prior sequencing using a QIAquick PCR Purification Kit (QIAGEN). Sequencing was carried out using an automated sequencer ABI3730XL in both directions. Sequence alignment was carried out using Clustal W (Larkin et al. 2007) and edited manually by using software 132 BioEdit v7.0.8.0 (Hall 1999). Distance matrix based on Kimura-2 parameter, and phylogenetic trees were built with MEGA 7 (Tamura et al. 2007). For identification purposes, we used all acanthocephalan sequences obtained from GenBank after sequence comparison and also the taxonomy browser of the Barcoding of Life Data System (Bold) http://www.boldsystems.org/index.php/TaxBrowser_Home.

To determine the epidemiological situation of this parasitic disease in the Algerian hedgehog, we analyzed the clinical records of all positive animals in a 66-month period. We calculated the prevalence of the disease in this hedgehog species that arrived at the recovery center. The association between age, body weight and sex, and the disease were analyzed by revising the clinical history of all positive animals. Information on the pharmacological treatments was applied; days of hospitalization, coinfections, and clinical signs were also included in this study.

Results

During a 66-month period (January 2013–June 2018), a total of 277 *A. algirus* specimens were necropsied after hospitalization and death at the Balearic wildlife recovery center. During these years, seven hedgehogs were found to be infected by unknown acanthocephalan larvae. The majority of the specimens were located in the peritoneal cavity (71.4%), while only two (28.6%) were found inside the intestine. The specimens in the peritoneum were encapsulated by surrounding tissue appearing more degraded than those found as free larvae in the intestine.

The larvae specimens were identified as acanthocephalan cystacanths as these showed the typical proboscis-containing hooks. The number of hooks rows varied from 16 to 20 with 11–14 hooks per each row. Other measurements such as the

length and width of the proboscis and the total length and width of the body are summarized in Table 1. The microscopic observation showed that the problem specimens contained reproductive organs. A close examination showed that these were similar to the ovarian balls reported for *P. cylindraceus* as described by Schmidt and Olsen (1964). The morphometric/morphological examination of the proboscis was consistent with this identification (S2).

We amplified and sequenced a 615-bp fragment within the *COI* gene region of two cystacanth specimens (GenBank accession numbers: MK300542 and MK300543 for specimens PC1 and PC2, respectively). The results of the BLAST nucleotide sequence similarity analysis showed that our problem specimens were 98–99% similar to *P. cylindraceus* (including the synonymous species *Plagiorhynchus transversus*). The Barcoding of Life Data System server failed to identify our problem specimens as we could not retrieve any results. We constructed a neighbor joining phylogenetic tree (Fig. 1). In addition to co-specific sequences, we also included sequences from all species resulting from the highly similar (Megablast) identification analysis: *Arhythmorhynchus frassoni* (EU189484.1) and *M. moniliformis* (AF416998.2). After alignment with other similar sequences in GenBank, the resulting amplicon was 547 bp long. Distance sequence matrix constructed with the Kimura-2 parameter algorithm showed that intraspecific distance was ≤ 2% while intergeneric distances ranged from 36.7 to 65.7% for *A. frassoni* and *M. moniliformis* respectively (Table 2). These results confirm the identity of our problem specimens as *P. cylindraceus*. Voucher specimens (VZOO2019-1 and VZOO201-2) are kept at the Museum of Zoology of the University of the Balearic Islands and are available for further studies.

In the 66-month study period, the overall prevalence of *P. cylindraceus* in *A. algirus* was 2.5% (3 males and 4 females). As it is shown in Table 3, the majority of infected hedgehogs were young specimens (body weight < 319 g) while only a single adult specimen (body weight = 426 g) was found positive for this parasite. All animals were given fluid replacement therapy with saline, and some of them received antibiotics, anti-parasitic treatments, and anti-inflammatory and bronchodilator drugs. All animals died within the first 3 days after internment, with the exception of one adult hedgehog, which survived for up to 11 days in hospitalization. Coinfection with lungworm helminths was found in all animals infected with *P. cylindraceus*. An enlarged spleen (splenomegaly) was observed in three of the positive hedgehogs (Table 3).

Table 1 Morphometric measurements in *P. cylindraceus* cystacanths from hedgehogs of this study

Character	*N*	Min.	Max.	mean ± SEM	SD	CV (%)
No. rows	6	16	20	17.33 ± 0,66	1.63	9.42
Hooks/row	3	11	14	12.33 ± 088	1.53	12.38
Proboscis length (mm)	3	0.79	1.1	0.93 ± 0,09	0.16	16.75
Proboscis width (mm)	6	0.23	0.35	0.28 ± 0,01	0.04	15.46
Body length (mm)	6	2.62	4.2	3.43 ± 0,30	0.75	21.74
Body width (mm)	6	0.8	1.5	1.10 ± 0,09	0.24	21.91

N: number of specimens, *SEM* standard deviation of the mean, *SD* standard deviation, *CV* coefficient of variation

Discussion

This study reports for the first time the infection of the acanthocephalan parasite *P. cylindraceus* in the Algerian hedgehog *A. algirus*. Although this hedgehog species has not been categorized as "vulnerable" at a global scale, it has disappeared in some Spanish regions where it was previously reported. Their populations seem to be stable in the Balearic archipelago, where the subspecies *Atelerix algirus vagans* is present (Alcover 2007). However, it has been the most common species of mammal hospitalized in the Wildlife Rehabilitation Centre at Balearic Islands during the 66-month study period.

From 2014, several acanthocephalan cystacanths were found inside the peritoneal cavity and intestine *of A. algirus*. Their overall morphology and location did not correspond with *M. moniliformis,* the only acanthocephalan reported for this species (Khaldi et al. 2012). Therefore, we conducted a more comprehensive examination of the specimens. The morphological identification of these larvae was impaired by the limited number of informative characters at the cystacanth stage, which were mainly restricted to the proboscis. The current keys for *P. cylindraceus* are based on the morphology of adult specimens (Amin et al. 1999). These characters have proved useful to distinguish other acanthocephalan species (Wayland 2010). However, in some cases, the number of hooks, hook rows, and proboscis dimensions can overlap among species (Lisitsyna 2010). The coefficient of variation values found in this study (Table 1) was even higher than those reported by Lisitsyna (2010). The total length of the body was consistent with measurements reported in the literature for the infective cystacanth of *P. cilindraceus* (Schmidt and Olsen 1964).

Mitochondrial markers, particularly the barcoding region of the *Cytochrome oxidase I* gene, are considered the "gold standard" for molecular identification in many taxa (Hebert et al. 2003a). However, its use in acanthocephalan taxonomy is still limited. We confirmed the identity of our problem specimens using this approach. The BLAST-nucleotide sequence comparison of the 615 bp amplicon showed that cystacanths were *P. cilindraceus*. This conclusion was based on the intraspecific sequence divergence (≤ 2%), which lies within the intraspecific range found in other acanthocephalans (1–5%) (Garcia-Varela and de Leon 2008). The DNA-barcoding

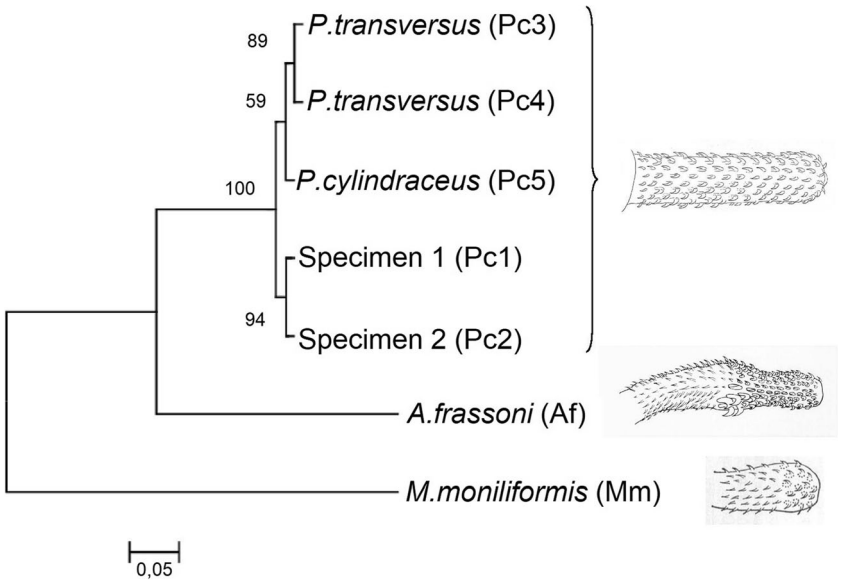

Fig. 1 Neighbor-joining tree based on 547-bp fragment of the *COI* region using the Kimura two-parameter substitution model. Numbers on branches are bootstrap values

approach combined with the morphological analysis of the proboscis have been effectively used to identify cystacanths in *Polymorphus brevis* (Alcantar-Escalera et al. 2013). This information was used by the authors to complete information on the parasite's life cycle and intermediate hosts. Using the *COI* gene region, Falla et al. (2015) reported two separate lineages in the primate acanthocephalan *Prosthenorchis elegans*, demonstrating that DNA barcodes may help in the detection of cryptic speciation in this phylum, as it has been reported in other taxa (Hebert et al. 2004). This study would have benefited from a larger dataset, including sequences from the remaining ten members of the *Plagiorhynchus* genus, that were not available in Gen Bank. This limitation has been reported in other acanthocephalan species, such as those from the *Corynosoma* genus (Waindok et al. 2018). The construction of a DNA-barcoding library for this phylum would allow the identification of immature specimens providing a baseline for epidemiological studies.

Table 2 Distance between pairs corrected by Kimura-2 parameter for the identification of *P. cylindraceus* cystacanths

	Pc1*	Pc2*	Pc3	Pc4	Pc5	Af
Specimen 1 (Pc1)*						
Specimen 2 (Pc2)*	0.007					
P. transversus (Pc3)	0.019	0.015				
P. transversus (Pc4)	0.019	0.015	0.000			
P. cylindraceus (Pc5)	0.022	0.019	0.011	0.011		
A. frassoni (Af)	0.367	0.360	0.357	0.357	0.364	
M. moniliformis (Mm)	0.657	0.662	0.652	0.652	0.657	0.770

Samples marked with (*) are those sequenced in this work

Pc Plagiorhynchus transversus/cylindraceus (synonyms) with accession numbers: NC029767 (Pc3), KT447549 (Pc4), and DQ089714 (Pc5); *Af Arhythmorhynchus frassoni* accession number EU189484; *Mm Moniliformis moniliformis*, accession number AF416998

The presence of ovarian balls inside the cystacanths was consistent with the molecular analysis and confirmed the identity of our specimens as *P. cylindraceus*. This is the only species of this genus that can develop reproductive organs at an immature stage (Schmidt and Olsen 1964). This parasite species has previously been found in other hedgehog species: *E. europaeus* and *E. romanicus* (Pfaffle et al. 2014). Khaldi et al. (2012) reported the presence of non-identified larval forms of an acanthocephalan in *A. algirus*. The absence of taxonomical informative characters did not allow these authors to arrive to further conclusions, but considering their location in the host, it is likely that these were in fact *P. cylindraceus*. The intestinal wall of young hosts is thinner than that of adults, increasing the risk of perforation by the acanthocephalan proboscis (Taraschewski 2000). Then, it is not surprising that the majority of affected hedgehogs were juvenile. Similar results were obtained by Skuballa et al. (2010) who found that infections by *P. cylindraceus* caused more severe cases in juvenile hedgehogs than in their older counterparts. Fatal cases of the disease, frequently associated with diarrhea and abdominal swelling have been reported by these authors in several European wildlife hospitals. Taking into consideration that *A. algirus vagans* is smaller than other European hedgehogs, it would be important to determine if the clinical manifestations are more severe in this species. Another interesting finding of this study is that all positive hedgehogs were co-infected by lungworms. Verminous pneumonia is a common cause of death in hedgehogs (Cousquer 2004). The infection by the acanthocephalan *P. cylindraceus* may be opportunistic, and penetrations of the peritoneum may

Table 3 General information and clinical manifestations recorded in hospitalized hedgehogs positive to the infection by *P. cilindraceus*

Age	Sex	Weight (g)	Coinfection	Treatment	Days in hospital	Other clinical manifestations
Juvenile	Female	154	Yes (lungworms)	MFT	2	Hypothermy, weakness, breathing difficulty, dehydration
Juvenile	Female	191	Yes (lungworms)	**	2	Bilateral pneumonia, hypothermy, weakness, breathing difficulty, dehydration, anemic spleen, ganglia inflammation, mucus in duodenum
Juvenile	Female	161	Yes (lungworms)	**	1	Bilateral pneumonia
Juvenile	Male	100	Yes (lungworms)	MFT	1	Breathing difficulties
Adult	Male	426	Yes (lungworms)	**	11	Bilateral pneumonia, splenomegaly
Juvenile	Female	296	Yes (lungworms)	**	3	Bilateral pneumonia, splenomegaly
Juvenile	Male	319	Yes (lungworms + myiasis)	MFT, antibiotics	0	Bilateral pneumonia, splenomegaly

**Maintenance fluid treatment (MFT), antibiotics, and anti-inflammatory, anti-parasitic, bronchodilator drugs

be the result of a compromised host immunity. Nevetheless, more studies are needed to arrive to firm conclusions. Hedgehogs are a source of several zoonoses in humans (Behr 2005). Although there are no reports of *P. cylindraceus* infections in humans, the fact that this species is opportunistic in its host preference, leads us to question whether this has the potential to infect humans or not. Acanthocephaliasis is rarely found in humans, but cases are most frequently reported in small children, after detection of adult worms or eggs in stools (Salehabadi et al. 2008). In the case of *P. cylindraceus*, infections may pass undetected since this parasite does not complete the life cycle in mammals and should be taken into consideration in areas where this is highly prevalent.

The results of this study show that the prevalence and the level of infestation of *P. cylindraceus* in *A. algirus* (2.5%) is much lower than that reported in European populations of *E. europaeus,* which has been reported higher than 40% in some regions of Germany and in the UK (Skuballa et al. 2010). It is important to emphasize that the prevalence values obtained in this study may differ from those found in nature, since our samples were obtained at a rehabilitation hospital. However, the studies conducted in Germany and the UK came also from hedgehog care centers. Low prevalence values of *P. cylindraceus* in hedgehogs have been associated with the recent colonization of this parasite in New Zealand (Skuballa et al. 2010). Although this may explain also the low prevalence found in *A. algirus*, other factors related to the host-parasite interaction may play an important role and should be taken into consideration. Studying the trend of this parasitic infection in areas where this was not previously reported is crucial to determine its expansion potential.

Acknowledgments We thank to all members of the COFIB for their collaboration and enthusiasm in the development of this study. We truly appreciate the collaboration of the members of the Cell Biology and Botany laboratories from sharing their molecular laboratory facilities. We would also like to thank the *SOIB JOVE* program for funding the contract of SD. Finally, we thank Ursula Paredes and Richard Abel for their thoughtful criticism to the manuscript.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

References

Alcantar-Escalera FJ, Garcia-Varela M, Vazquez-Dominguez E, de Leon GPP (2013) Using DNA barcoding to link cystacanths and adults of the acanthocephalan *Polymorphus brevis* in central Mexico. Mol Ecol Resour 13:1116–1124

Alcover JA (2007) *Atelerix algirus* (Lereboullet, 1842). In: Palomo GJ, L.J, Blanco JC (eds) Atlas y libro rojo de los mamíferos terrestres de España. Dirección General de Conservación de la Naturaleza-SECEM-SECEMU, Madrid, pp 83–85

Amin OM, Canaris AG, Kinsella JM (1999) A taxonomic reconsideration of the genus *Plagiorhynchus* s. lat. (Acanthocephala : Plagiorhynchidae), with descriptions of South African *Plagiorhynchus* (*Prosthorhynchus*) *cylindraceus* from shore birds and *P.* (*P.*) *malayensis*, and a key to the species of the subgenus *Prosthorhynchus*. J Helminthol Soc Wash 66:123–132

Behr M (2005) Hedgehog zoonoses. Emerg Infect Dis 11:1146–1146

Ching HL, Leighton BJ, Stephen C (2000) Intestinal parasites of raccoons (*Procyon lotor*) from southwest British Columbia. Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire 64:107–111

Coady NR, Nickol BB (2000) Assessment of parenteral *Plagiorhynchus cylindraceus* (Acanthocephala) infections in shrews. Comp Parasitol 67:32–39

Cousquer G (2004) Analysis of tracheal sputum for diagnosing and monitoring verminous pneumonia in hedgehogs (*Erinaceus europaeus*). Vet Rec 154:332–333

Edmonds SJ (1989) A list of Australian Acanthocephala and their hosts. Records of the South Australian Museum 23:127–133

Falla AC, Brieva C, Bloor P (2015) Mitochondrial DNA diversity in the acanthocephalan *Prosthenorchis elegans* in Colombia based on *cytochrome c oxidase I* (*COI*) gene sequence. International Journal for Parasitology-Parasites and Wildlife 4:401–407

Ferrer D, Molina R, Castella J, Kinsella JM (2004) Parasitic helminths in the digestive tract of six species of owls (Strigiformes) in Spain. Vet J 167:181–185

Florescu BI (1984) Survey of Acanthocephala in Romania. Travaux du Muséum d'Histoire Naturelle Grigore Antipa 25:7–45

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial *Cytochrome c Oxidase* subunit *I* from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3: 294–299

Garcia-Varela M, de Leon GPP (2008) Validating the systematic position of *Profilicollis meyer*, 1931 and *Hexaglandula petrochenko*, 1950 (Acanthocephala : Polymorphidae) using *Cytochrome C oxidase* (COX 1). J Parasitol 94:212–217

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

Hebert PDN, Ratnasingham S, deWaard JR (2003a) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc R Soc London Ser B-Biol Sci 270:S96–S99

Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003b) Biological identifications through DNA barcodes. Proc R Soc B-Biol Sci 270:313–321

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc Natl Acad Sci U S A 101:14812–14817

Khaldi M, Torres J, Samso B, Miquel J, Biche M, Benyettou M, Barech G, Benelkadi HA, Ribas A (2012) Endoparasites (helminths and coccidians) in the hedgehogs *Atelerix algirus* and *Paraechinus aethiopicus* from Algeria. Afr Zool 47:48–54

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and clustal X version 2.0. Bioinformatics 23:2947–2948

Lisitsyna O (2010) Morphological variability of *Plagiorhynchus* (*Prosthorhynchus*) *cylindraceus* (Acanthocephala, Plagiorhynchidae) and its importance in assessment of taxonomy structure of the subgenus. Vestnik zoologii 44:e-35–e-44

Morales A, Rofes J (2008) Early evidence for the Algerian hedgehog in Europe. J Zool 274:9–12

Pfaffle M, Bolfikova BC, Hulva P, Petney T (2014) Different parasite faunas in sympatric populations of sister hedgehog species in a secondary contact zone. PLoS One 9:e114030

Salehabadi A, Mowlavi G, Sadjjadi SM (2008) Human infection with *Moniliformis moniliformis* (Bremser 1811) (Travassos 1915) in Iran: another case report after three decades. Vector-Borne and Zoonotic Diseases 8:101–103

Schmidt GD, Olsen OW (1964) Life cycle and development of *Prosthorhynchus formosus* (Van Cleave, 1918) Travassos, 1926, an acanthocephalan parasite of birds. J Parasitol 50:721–730

Skuballa J, Taraschewski H, Petney TN, Pfaffle M, Smales LR (2010) The avian acanthocephalan *Plagiorhynchus cylindraceus* (Palaeacanthocephala) parasitizing the European hedgehog (*Erinaceus europaeus*) in Europe and New Zealand. Parasitol Res 106:431–437

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Taraschewski H (2000) Host-parasite interactions in Acanthocephala: a morphological approach, pp. 1-179. In J. R. Baker, R. Muller and D. Rollinson (eds.), Adv Parasitol, Vol 46

Waindok P, Lehnert K, Siebert U, Pawliczka I, Strube C (2018) Prevalence and molecular characterisation of Acanthocephala in pinnipedia of the North and Baltic Seas. Int J Parasitol-Parasites Wildl 7:34–43

Wayland MT (2010) Proboscis profiler: a tool for detecting acanthocephalan morphotypes. Syst Parasitol 76:159–167