

Acanthocephala Species of Mammals in Türkiye and A New Species Record from Foxes

Türkiye'de Memelilerde Acanthocephala Türleri ve Tilkilerden Yeni Bir Tür Kaydı

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ABSTRACT

Acanthocephalosis is a zoonotic parasitic infection of vertebrates. The phylum Acanthocephala contains nearly 1500 acanthocephalan species. The Archiacanthocephala class is observed in terrestrial habitats and usually has a large, spineless trunk. Acanthocephalans are parasitic worms that use insects as intermediate hosts in their two-host life cycles. Insects, millipedes, and crustaceans in terrestrial areas serve as intermediate hosts and birds and mammals as definitive hosts. Acanthocephalans collected from the red fox (*Vulpes vulpes*) found dead on the road to Sarıkamış-Kars in 1995 and stored in formaldehyde were kept in Ondokuz Mayıs University Faculty of Veterinary Medicine, Veterinary Parasitology Laboratory Museum until 2023 after our parasitological study found an infected red fox with *Pachysentis* sp. This study provides the anatomy of the acanthocephalans and the laboratory practice necessary for a good and reliable diagnosis. This study reports a new species, *Pachysentis* sp., of acanthocephalan (thorny-headed worm) found in red foxes for Türkiye. On the basis of relevant articles, we have created a key to Acanthocephala species occurring in mammals.

Keywords: Acanthocephala, *Pachysentis*, Türkiye, *Vulpes vulpes*

ÖZ

Acanthocephalosis omurgalıların zoonotik parazitik bir enfeksiyonudur. Acanthocephala kökü yaklaşık 1500 acanthocephalan türü içerir. Archiacanthocephala sınıfı karasal habitatlarda özgü görülür ve genellikle büyük, dikensiz bir gövdeye sahiptirler. Acanthocephalalar, iki konaklı yaşam döngülerinde böcekleri ara konak olarak kullanan parazitik canlılardır. Karasal alanlarda böcekler, kırkayaklar ve kabuklular arakonak, kuşlar ve memeliler ise son konak olarak görev yapar. Sarıkamış-Kars yolu üzerinde 1995 yılında ölü bulunan kızıl tilkiden (*Vulpes vulpes*) toplanan ve formaldehit içerisinde saklanan Acanthocephalalar, 2023 yılına kadar Ondokuz Mayıs Üniversitesi Veteriner Fakültesi, Veteriner Parazitoloji Laboratuvar Müzesi'nde muhafaza edilmiştir, parazitolojik çalışmamızda *Pachysentis* sp. olarak teşhis edilmiştir. Bu çalışma, acanthocephalan'ların anatomisini ve iyi ve güvenilir bir teşhis için gerekli laboratuvar uygulamalarını vermektedir. Bu çalışma, Türkiye için kızıl tilkilerde görülen yeni bir akantosefalan (diken başlı solucan) türünü, *Pachysentis* sp., bildirmektedir ve memelilerde görülen acanthocephalan türleri için bir anahtar içermektedir.

Anahtar Kelimeler: Acanthocephala, *Pachysentis*, Türkiye, *Vulpes vulpes*

INTRODUCTION

The phylum Acanthocephala contains four classes and nearly 1500 acanthocephalan species (1). Archiacanthocephala class is observed in terrestrial habitats, and Eoacanthocephala, Palaeacanthocephala, and Polyacanthocephala classes are kept in aquatic habitats. Class Archiacanthocephala is usually a large, spineless trunk. Cement glands usually have eight multinucleate. Insects, millipedes, and crustaceans in

terrestrial areas serve as intermediate hosts, and birds and mammals as definitive hosts (2).

Archiacanthocephala consists of four orders. The order Apororhynchidae, with one genus and seven species, occurs entirely in birds (3,4). The order Moniliformida has three genera. *Australiformis* contains one species, *Australiformis semoni*, and has a localised distribution in Australia and New Guinea marsupials (5). *Promoniliformis* contains one species, *Promoniliformis ovocristatus*, and has a localised distribution in the



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Afrotropical region in tenrecs (6). *Moniliformis* contains eighteen species and occurs in mammals, including humans. The order Gigantorhynchidae has three genera (4). *Gigantorhynchus* comprises six species, all found in mammals in South America and baboons in Africa (7). *Intraproboscis* has one specie, *Intraproboscis sanghae*; this acanthocephalan species is found in mammals in Africa, and *Mediorhynchus* contains at least 60 acanthocephalan species from birds worldwide (7). Oligacanthorhynchida contains twelve genera, including *Macracanthorhynchus*, *Pachysentis*, etc., and numerous species (4).

For a satisfactory and comfortable morphological diagnosis of acanthocephalans, it is necessary to have a good command of their anatomy. Their bodies are generally divided into the proboscis, the neck and the trunk. The proboscis can be short and stubby, cylindrical with few hooks or cylindrical with many hooks (Figure 1). The proboscis has hooks and hooks' roots. The hooks and the roots are essential for identification. The neck is the unarmed part of the trunk immediately following the most posterior circle of hooks on the proboscis. It may be virtually non-existent or very long and well-developed. The proboscis receptacle, the lemnisci and the reproductive system are in the trunk. The proboscis receptacle allows the proboscis to be pulled in and out of the trunk and has a single or double wall. A pair of lemniscus arises at the base of the neck and is thought to have an absorptive function. The lemniscus may be of equal or unequal length, short or very long, with or without a nucleus, and of various shapes. The female reproductive system comprises the gonads, where the ovarian balls develop and produce the oocytes and the eggs after fertilisation. The efferent duct of the female system contains the uterine bell, the uterus and the vagina. The vaginal sphincter and the genital opening are located posterior to the trunk and all the genital organs. The male reproductive system generally consists of two testes, a seminal vesicle, cement glands, a cement reservoir, a Saeftigen's pouch, a copulatory bursa and a penis. The testes are spheroid-ovoid or elongated, tubular, usually found in tandem. The cement glands can be four, six or eight in number and ovoid, piriform, elongated or tubular (8,9).

The life cycle of acanthocephalans generally consists of an intermediate host and a final host. Adult and mated female acanthocephalans lay eggs in the intestine of vertebrates. The eggs are excreted together with the faeces of the host. A proper intermediate host takes up the acanthor in the egg. The acanthor emerges from the egg, penetrates the intestine of the intermediate host and passes into the haemocoel. Within the haemocoel, the acanthor first develops into acanthellae and then into cystacanth, an infective form for the final hosts. The definitive hosts become infected by taking the infective form into the intermediate host. Sometimes cystacanth can be taken up by paratenic intermediate hosts. In such a case, the cystacanth

penetrates the intestinal wall of the paratenic intermediate host and enters the mesentery, where its cysts. Suitable end-hosts can complete the infection by eating paratenic arachnids with cystacanths in the mesentery (10,11). If another definitive host eats an infected host, acanthocephalans can infect the new host. This type of transmission is called post-cyclic transmission (12).

MATERIALS AND METHODS

In 1995, a red fox (*Vulpes vulpes*) was found dead in a traffic accident on the Kars-Sarıkamış Road and kept in formaldehyde to prevent putrefaction during transport. Then necropsy was performed, and the acanthocephalan specimens found were placed in formaldehyde again. Until 2023, they were kept in Ondokuz Mayıs University Faculty of Veterinary Medicine, Veterinary Parasitology Laboratory Museum. Some specimens were dissected to understand their morphological characteristics. Other samples were prepared with Semichon carmine following the procedure given below. Then the parasites were identified using the morphological features under a Nikon Eclipse 80i light microscope. Special morphological characters were measured and photographed using the MDX4 - T Mshot camera integrated into the microscope and then drawn with Adobe Illustrator 2020 (Figure 2).

The following procedure is used to prepare the preparation from acanthocephalans (8,13). This procedure is based on relevant literature and laboratory experience.

- The collected acanthocephalans should be kept in cold water for 15 minutes to 3 hours. The proboscis will evert for about 50% of the acanthocephalan specimens. The bill must be out for species identification.
 - Transfer to 70% ethanol (At least 48 hours).
 - Transfer Acanthocephalan from 70% ethanol into Semichon's stain (2-4-hour average).
 - Transfer to 70% ethanol (60 min).
 - The over-dye is destaining in 1% acid alcohol. Acanthocephalan specimens are kept in the solution until they turn pinkish-red colour. Depending on the type of stain and acanthocephalan, the waiting time varies between 1 minute and 30 minutes.
 - Transfer to 1% basic alcohol. Acanthocephalan specimens are kept in the solution until they turn blue. Depending on the type of stain and acanthocephalan, the waiting time varies between 1 minute and 30 minutes.
 - Transfer to 70% ethanol. Acanthocephalan specimens are kept in the solution for at least 30 minutes.
 - Transfer to 95% ethanol. Acanthocephalan specimens are kept in the solution for at least 60 minutes.
 - Transfer to 96-100% ethanol. Acanthocephala specimens are kept in the solution for at least 60 minutes.
 - Transfer to methyl benzoate. Acanthocephalan specimens are kept in the solution for at least 60 minutes. In this step, the Acanthocephalan specimens become transparent, and the internal structure is visible.
 - Mount in Canada balsam.
- ***1% acid alcohol, 70% ethanol with 1 mL 1 N HCl/10 mL.
 ***1% basic alcohol, 70% ethanol with 1 mL 1 N NaOH or ammonia/10 mL.
 ***Xylene can also be preferred instead of methyl benzoate.

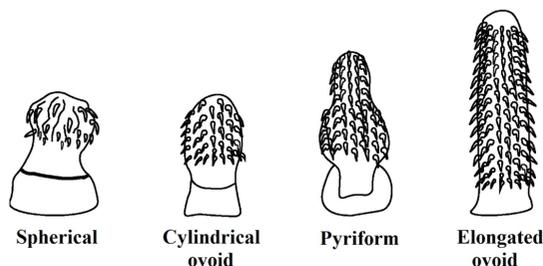


Figure 1. Line drawing of variations of the proboscis (original)

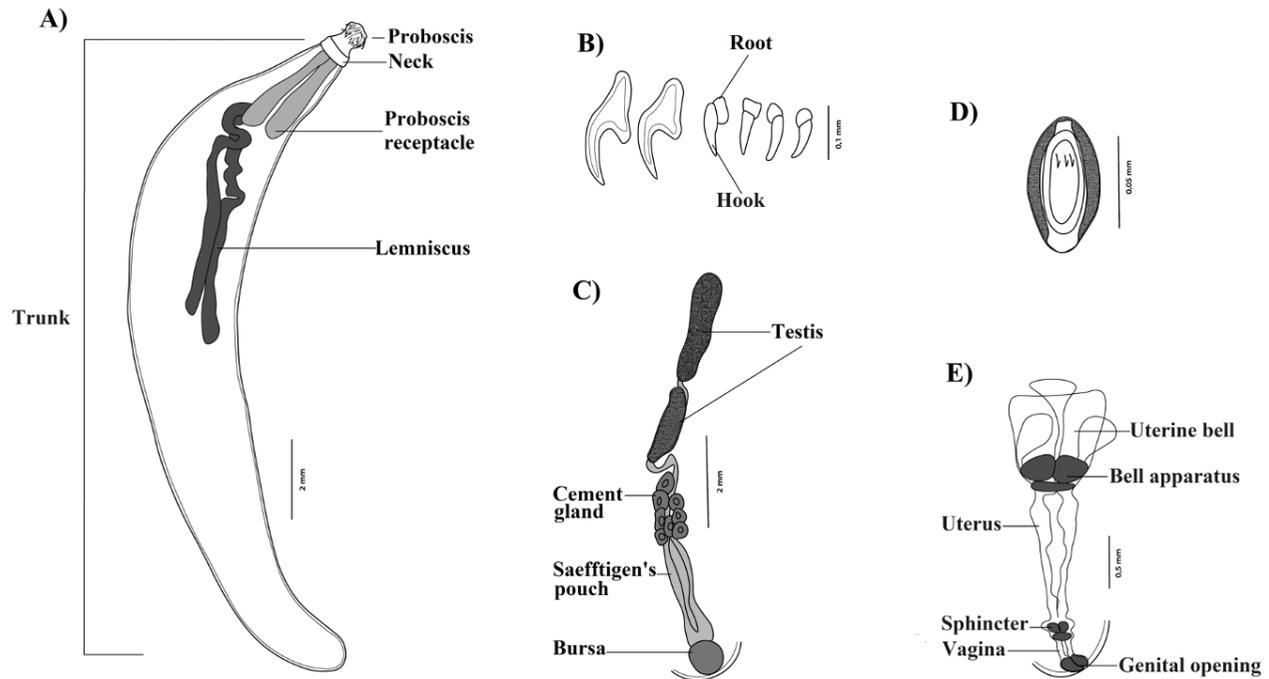


Figure 2. *Pachysentis* sp. was recovered in a red fox. A) *Pachysentis* sp. bar: 2 mm, B) Dissected proboscis, bar: 0,1 mm, C) *Pachysentis* sp in formaldehyde before being dissected, bar: 2 mm (original)

RESULT AND DISCUSSION

Acanthocephalans are common in vertebrates worldwide, including humans (Table 1). Most human cases are due to the consumption of fish or insects. Although human cases have been reported in geographically close countries, they have not yet been reported in Türkiye. In mammals from Türkiye, *Moniliformis moniliformis* was reported in rodents (*Rattus norvegicus*) in 1963 and 1975 (14,15). *Macracanthorhynchus hirudinaceus* was reported in domestic pigs (*Sus scrofa dom.*) in 1964 and wild pigs (*Sus scrofa*) in 1965 (16,17). *Macracanthorhynchus hirudinaceus* was reported in red foxes (*V. vulpes*) in 1965, and its prevalence was given 43.1% (18). *Prosthorhynchus* was reported in hedgehogs (*Erinaceus europaeus*) in 1970 (19). A study was conducted in Central Anatolia and Thrace, and it was reported that acanthocephalan eggs were found in 37 of 409 red foxes faeces in 2019 (20). With this study, *Pachysentis* sp. is reported for the first time in Türkiye, and a new genus has been added to the acanthocephalan found in mammals in Türkiye.

Amin (4) published a taxonomy of the genus *Pachysentis*, including *P. angolensis* (Golvan, 1957) Schmidt, 1972 (syn. *Oncicola angolensis* Golvan, 1957), *P. canicola* Meyer, 1931 (type species) (fide Van Cleave, 1953), *P. dollfusi* (Machado Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis dollfusi* Machado Filho, 1950], *P. ehrenbergi* Meyer, 1931, *P. gethi* (Machado Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis gethi* Machado Filho, 1950], *P. lenti* (Machado Filho, 1950) Schmidt, 1972 [syn. *Prosthenorchis lenti* Machado Filho, 1950], *P. procumbens* Meyer, 1931, *P. procyonis* (Machado Filho, 1950) Schmidt, 1972. *Pachysentis* sp. was reported in red foxes from Greece (21). *Pachysentis angolensis* was reported in the side-striped jackal (*Lupulella adusta*) from the Angolo (22). *Pachysentis*

canicola was reported in red fox and the golden jackal (*Canis aureus*) from Iran, in the maned wolf (*Chrysocyon brachyurus*), the striped skunk (*Mephitis mephitis*), the western diamondback rattlesnake (*Crotalus atrox*) from the United States of America (23-27). *Pachysentis dollfusi* was reported in the common brown lemur (*Eulemur fulvus*) from Madagascar, Africa (28). In Egypt, *P. ehrenbergi* was reported in the long-eared hedgehog (*Hemiechinus auratus*) and red fox (22,29). *Pachysentis gethi* was reported in the lesser grison (*Galictis cuja*), the greater grison (*Galictis vittata*) and the tayra (*Eira barbara*) from Brazil (30). In Brazil, was reported *P. lauroi* in the south american coati (*Nasua nasua*), *P. lenti* in the white-headed marmoset (*Callithrix geoffroyi*), *P. procyonis* in the crab-eating raccoon (*Procyon cancrivorus*), *P. rugosus* in Azaras's capuchin (*Sapajus cay*), and *P. septemserialis* in the black tamarin (*Saguinus niger*) (31-34). *Pachysentis procumbens* was reported in red fox from Egypt (22). Based on these cited references, the hosts for *Pachysentis* species are primates and carnivores within geographic areas of Africa, Asia, Europe and North, Central and South America.

A key to the genus of mammals Acanthocephalans (22,34,35):

1. Anterior region of the body bearing a proboscis upon which hooks or spines are usually arranged in parallel alternating rows-
2. The region between the body proper and the proboscis (the neck) is elongated and cylindrical, except in the area adjacent to the proboscis, where a conspicuous spherical enlargement occurs-Genus *Pomphorhynchus*.
2. Region behind the proboscis is variously modified but never bearing a spherical enlargement followed by a narrow, elongated neck. The body properly never bears spines (Demonstration of minute spines on the anterior part of the body in cleared specimens of some genera requires careful light adjustment)-3 or 4

Table 1. Cases of human acanthocephaliasis published in the literature.

Species	Country	Reference
<i>Acanthocephalus rauschi</i>	Alaska	(22)
<i>Bolbosoma cf. capitum</i>		(36)
<i>Bolbosoma nipponicum</i>		(37)
<i>Bolbosoma</i> sp.	Japan	(38)
		(39)
		(40)
		(41)
<i>Corynosoma strumosum</i>	Alaska	(41)
<i>Corynosoma cf. validum</i>	Japan	(42)
<i>Corynosoma villosum</i>		(43)
<i>Macracanthorhynchus hirudinaceus</i>	China	(44)
		(45)
	Thailand	(46)
		(47)
<i>Macracanthorhynchus ingens</i>	USA	(48)
		(49)
		(50)
		(51)
		(52)
<i>Macracanthorhynchus sp.</i>	Papua New Guinea	(53)
<i>Moniliformis moniliformis</i>	Australia	(54)
	Iran	(55)
		(56)
		(57)
		(58)
		(59)
	(60)	
	Iraq	(61)
	Nigeria	(62)
		(63)
	Zimbabwe	(64)
	Saudi Arabia	(65)
Australia	(66)	
USA	(67)	
	(68)	
<i>Plagiorhynchus</i> sp.	United Kingdom	(69)
<i>Pseudoacanthocephalus bufonis</i>	Alaska	(41)

3. Body just behind the proboscis is swollen, closely set and serving as an accessory organ of fixation. Proboscis medium length. Parasitic as adults in mammals and birds-Genus *Corynosoma*.

Body spines are arranged as a collar near the anterior extremity and behind. This is placed in 18-23 cross-rows of closely set spines, the rows separated by a considerable area devoid of spines-Genus *Serrasentis*.

4. Subcuticula bearing a few rounded giant nuclei, prominent in stained whole mounts and sections and their location frequently recognisable in preserved specimens as minute elevations of the body surface.

Wall of proboscis receptacle with but a single muscular layer. The cement gland of males is a single syncytial mass with a few giant nuclei. Family Neoechinorhynchidae-5 or 8

Proboscis globular, bearing three circles of hooks-5 or 7

5. Six hooks in each circle of hooks upon proboscis-Genus *Neoechinorhynchus*.

More than six hooks in each circle-6

6. Eight hooks in each circle-Genus *Octospinifer*.

Twelve hooks in each circle-Genus *Gracilisentis*.

7. Proboscis bearing more than three circles of hooks-8

Twenty or more circles of hooks. Subcuticular nuclei are all in the sagittal plane-Genus *Tanaorhampus*.

Eight circles of hooks upon proboscis. Giant nuclei of subcuticular are not all in the sagittal plane-Genus *Pandosentis*.

8. With eight cement glands. Parasitic in birds and mammals-9

The proboscis receptacle is a closed muscular sac with retractors through the posterior extremity. The outer layer of the receptacle is in spiral bands. Hooks are small and simple, each with a single posteriorly directed root. Parasitic in mammals. Body evidence of pseudo-segmentation-Genus *Moniliformis*.

9. Parasitic in mammals-10

Parasitic in birds.

10. Reproductive organs of males occupy more than one-half the length of the body cavity. Lemnisci frequently in contact testes-11

Proboscis is provided with a crown of a few circles of strong crowded so that they may have the appearance of a single and behind this crown a region of some length with closely set fine spines-Genus *Gigantorhynchus*.

Proboscis with several circles of strong hooks. In intestine marsupials and edentates-Genus *Hamanniella*.

11. Lemnisci is relatively short and flat. Testes considerably cement glands-Genus *Macracanthorhynchus*.

Lemnisci is relatively long and subcylindrical. They were removed from cement glands-Genus *Echinopardalis*.

The eight cement glands are not definite-12

Cement glands are almost spherical. Lemnisci is cylindrical, more than three-fourths the length cavity-Genus *Oncicola*.

Cement glands are closely crowded together, and a festoon surrounds the neck-Genus *Prosthenorchis*.

Cement glands are closely crowded together-Genus *Pachysentis*.

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***Authorship Contributions**

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