

Original Article

## Ultrastructure and elemental depositions of hooks in *Centrorhynchus* cf. *aluconi* (Acanthocephala: Polymorphida)

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### Abstract

*Centrorhynchus* are an acanthocephalan parasite, also known as a thorny-headed worm, which infects many avian and reptile species. They are dominated by hook-rows on their proboscis. However, microstructures and elemental profiles of the hooks are unclear for this worm. The aims of this study were to report the ultrastructure and elemental deposition of hooks in *C. cf. aluconi* collected from Chinese ratsnakes by using FESEM-EDX and TEM. Hook surfaces show narrow folds alternating with grooves longitudinally. However, surfaces of proboscis were quite smooth. Ultrastructure of the hook layers consisted of the first and second outer hook layers, and the first, second, and third inner layers. Hook surfaces are composed of sulfur ( $15.88 \pm 3.01$  of weight %), calcium ( $6.03 \pm 3.83$ ), phosphorus ( $13.73 \pm 2.89$ ), and others. Inside hooks consist of sulfur ( $24.81 \pm 4.36$ ), calcium ( $7.16 \pm 1.59$ ), phosphorus ( $11.86 \pm 5.20$ ), and others. Sulfur was predominant on the hook surface and the outer hook layer but not in the inner layer. Calcium had a high composition in the inner layer. These results may offer important data for the identification of *C. aluconi* and the understanding of the ultra-architecture and mineral profiles of hooks in this species.

**Keywords:** *C. cf. aluconi*, thorny-headed worms, ultrastructure, elements

### 1. Introduction

In acanthocephalans (thorny-headed worms or spiny-headed worms), the characteristics of the hooks and hook-rows on the proboscis are one of the most important morphologies for identifying a genus or species, including *Centrorhynchus* (McDonald, 1988). *Centrorhynchus* (Lühe, 1911) belongs to Centrorhynchidae in the order of Polymorphida (Amin, Crompton, & Brent, 1985), which have more than fifty recognized species (Amin, 2013). *Centrorhynchus* mainly infects the intestinal tracts of birds and reptiles, including amphibian and mammalian species (Choi *et al.*, 2010; Richardson, Smales, Ghorbani, & Halajian, 2017). Examples of these birds include

*C. aluconis* in Ural owls (*S. uralensis*), *C. buteonis* in common buzzards (*Buteo buteo*), and *C. globirostris* in pheasant crows (*Centropus sinensis*) (Amin, Heckmann, Wilson, Keele, & Khan, 2015; Komorová, Špakulová, Humíková, & Uhrín, 2015). In reptiles, there researchers have found *Centrorhynchus* sp. in tiger keelback snakes (*Rhabdophis tigrinus*) (Choi *et al.*, 2010) and anolis lizards (Polychrotidae) (Goldberg, Bursey, & Cheam, 1998) and *C. sindhensis* in Indian cobras (*Naja naja*) (Khan, Khatoon, & Bilqees, 2002). In amphibians, an infested example was reported for *Centrorhynchus* in anurans (Smales, 2007), in toads (*Eupsophus*) (Torres & Puga, 1996), and in burrowing toads (*Rhinella fernandezae*) (Santos & Amato, 2010). In mammals, Richardson, Smales, Ghorbani, and Halajian (2017) reported *Centrorhynchus* in the intestines of stray dogs in Qom Province, Iran. In addition, infections of *Centrorhynchus* were recorded in cats and banded Mongooses (*Mungos mungo*) (Baker, Lange, Verster, & van der Plaat, 1989; Halajian, Smales, Tavakol, Smit, & Luus-Powell, 2018).

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Considering the above documents, *Centrorhynchus* are endoparasites that appear in many none-mammalian and mammalian vertebrates. Therefore, this study of the micro-structural and elemental compositions of hook-rows may yield important data to help with further diagnoses and increase our understanding of the biological processes in *Centrorhynchus*.

## 2. Materials and Methods

*Centrorhynchus* samples were collected in March 2018 from six intestinal tracts of Chinese ratsnakes (*P. korros*) at agricultural and rural areas in Nakhon Nayok Province. These snakes were accidentally trapped in fish nets and killed by local people in order to protect their poultry and livestock. Spiny-headed worms were washed with 0.9% normal saline and were selected when fresh and living. Worms were identified as *C. cf. aluconi* via the key to acanthocephala (McDonald, 1988). Six specimens were separated via FESEM-EDX (field emission scanning electron microscopy with energy dispersive X-ray spectroscopy) analysis, and six worms were used for TEM (transmission electron microscopic) analysis.

After *C. cf. aluconi* were fixed in the hot formaldehyde solution, they were re-fixed in 2.5% glutaraldehyde fixative in 0.1 sodium cacodylate buffer (pH 7.4) at 4 °C for 6 h. the specimens were washed 3 times with 0.05 M sodium cacodylate buffer, post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for one hour, washed three times with distilled water, and dehydrated with a graded series of ethanol. After dehydration, they were dried in a Hitachi HCP-2 critical point drying apparatus. All specimens were mounted on aluminum stubs by carbon conductive tape, coated with gold particles in a SPI-Model sputter coater for 4 min, and viewed using a SEM-HITACHI SU-8010 operating at 15 kV. X-ray elemental analysis was performed by using XFlash 6 detector under a high-vacuum mode. The accelerating voltage was applied at 20 kV for EDX images, with 60 s for the counting time. Twelve four hooks from six proboscides were examined randomly.

Four worms were transferred into a Bouin's fixative solution overnight and were then stored in 70% ethanol. The specimens were dehydrated through a graded ethanol series and embedded in paraffin. Specimens were serially cross-sectioned into 6 µm thick slices using a microtome (Leica RM125). Sections were stained with hematoxylin and eosin solution and were examined under a light microscope (Olympus BX51).

For TEM, the fixation of *C. cf. aluconi* in a glutaraldehyde and osmium tetroxide solution was similar to that of the SEM procedure. After dehydration, the specimens were transferred to propylene oxide and epoxy resins (Araldite-502) for infiltration and were incubated at 45 °C. The resin-embedded specimens were sectioned thinly, stained with uranyl acetate, re-stained with lead citrate on a formvar 300-mesh with carbon-coated Cu grids, and imaged by a JEOL JEM-1400 series at 100 kV.

## 3. Results

In the SEM micrographs of *C. cf. aluconi*, surfaces of hooks and proboscides were quite smooth (Figure 1A, B). In contrast, the surface of hooks exhibited folds alternating with the narrow grooves longitudinally along the highly magnified image (Figure 1C). The surfaces of the folds were smooth.

Folds were observed to branch in some areas of the hook's surfaces. The surfaces of the proboscides were slightly puckered (Figure 1D). The surfaces of the worm's body without the sack were rough compared with the surfaces of the hooks and proboscides (Figure 1E, F). From a histological view, the outer hook layers were thick and almost transparent, with stout hook roots embedded in syncytial tegument. The striped layer of the tegument was thin and covered in a syncytial tegument (2A).

In the TEM investigation, the hooks were composed of five layers, as follow: the first outer hook layer, the second outer hook layer, and the first inner layer, then the second inner layer and the third inner layer. The first outer hook layer was compacted and seemed to be hard. Folds and grooves covered this layer. The second outer hook layer had many pits and electron dense materials. The first inner layer was irregular, thick, and sub-positioned the second outer layer near a tip area of the hook but was thin near the base of the hook. In contrast, the second inner layer was thin, wrinkled, and more compact than the first inner layer. In the third inner layer, there were electron dense materials with intermediate filaments, and this layer was thicker than the other layers (Figure 2B-F).

For the FESEM-EDX analysis, the hook surfaces were composed of carbon (C) ( $33.05 \pm 5.89$  of weight %), nitrogen (N) ( $16.47 \pm 4.80$ ), oxygen (O) ( $14.214 \pm 2.96$ ), phosphorus (P) ( $13.73 \pm 2.89$ ), sulfur (S) ( $15.88 \pm 3.01$ ), calcium (Ca) ( $6.03 \pm 3.83$ ), magnesium (Mg) ( $0.65 \pm 0.91$ ), and sodium (Na) ( $0.63 \pm 0.14$ ). The inside hooks consisted of C ( $29.8 \pm 6.70$ ), N ( $12.7 \pm 4.86$ ), O ( $11.71 \pm 6.15$ ), S ( $24.81 \pm 4.36$ ), Ca ( $7.16 \pm 1.59$ ), P ( $11.86 \pm 5.20$ ), Mg ( $1.32 \pm 1.30$ ), and Na ( $0.59 \pm 0.31$ ) (Figure 3A). C, N, and O were predominant elements of hooks and were found on the surface and inside (Figure 3B-G). However, Na was also detected on the surface

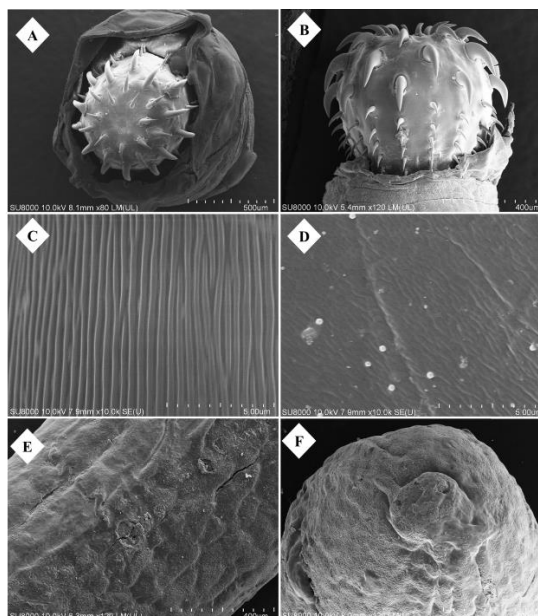


Figure 1. Scanning electron micrographs of *C. cf. aluconi*. Top view (A) and lateral view (B) of the proboscis armament with the hooks. A high magnification of the hook's surface (C), proboscis, body without sack (E), and posterior end without sack (F).

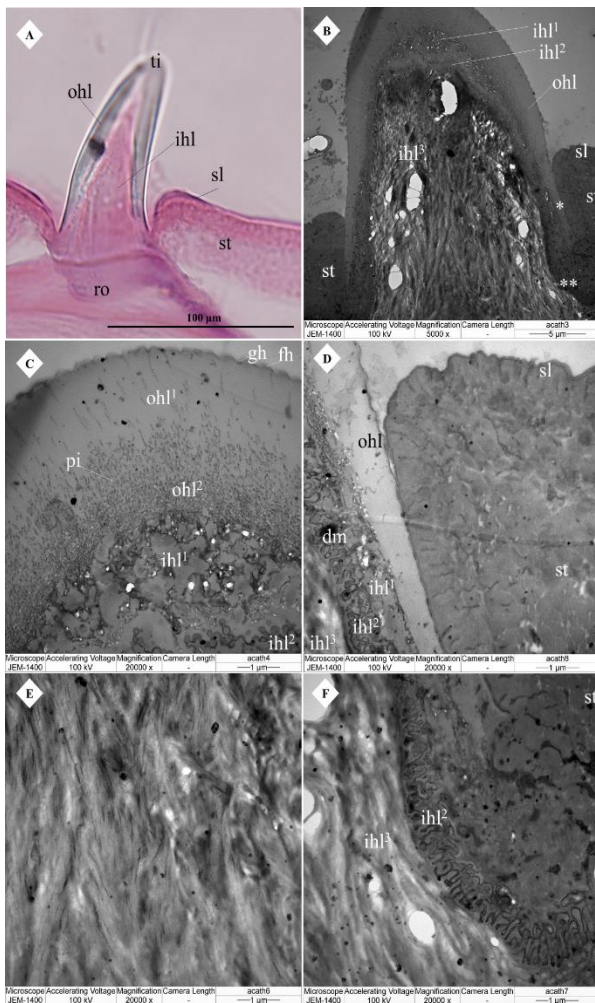


Figure 2. Histological and ultrastructural investigations of the hook. The histological section of the hook stained by H&E (A). Overview of the ultrastructure of the hook (B). Tip of the hook (C). Lateral area of the hook (D). High magnification of the third inner layer (E). High magnification of Fig B on the area is in double asterisks (F). The first outer hook layer (ohl<sup>1</sup>), the second outer hook layer (ohl<sup>2</sup>), the first inner hook layer (ihl<sup>1</sup>), the second inner hook layer (ihl<sup>2</sup>), the third inner hook layer (ihl<sup>3</sup>), the fold (fh) and groove (gr), the pit (pi), the electron dense material (de), the hook root (ro), the syncytial tegument (st), the striped layer (sl), and the tip (ti).

and inside of the hooks, but the signal was weak (Figure 3H). Mg was distributed in the outer and inner layer, while the surface had a low intensity (Figure 3I). P was detected in the surface and inner layer, but the signal was low in the outer layer (Figure 3J). S in the outer layer had a more predominant intensity than that on the surface. However, its intensity was weak in the inner layer (Figure 3K). Ca was found on the surface and inside the hooks. The inner layers of the hooks were dominated by Ca but not in the outer layer (Figure 3L). It should be noted that the microstructure, ultrastructure, and chemical profiles of each sample in the hook rolls were not different and did not exhibit sexual dimorphism. Figures displayed in the results were selected as representative of the replicated samples.

#### 4. Discussion

First, we need to introduce the findings for *C. cf. aluconi* in Chinese ratsnakes from Thailand. These findings correspond to the description of *C. aluconi* collected in wild waterfowls in the report of McDonald (1988) (merganser: *Mergus cucullatus*). Moreover, the *C. cf. aluconi* in this study was similar to the morphological description of *C. aluconi* in the small intestine of the *S. aluco* (Dimitrova & Gibson). *C. aluconi* were also found in birds of prey (e.g., *S. uralensis* and *S. aluco*) (Ewald & Crompton, 1993; Komorová, Špakulová, Hurnková, & Uhrín, 2015). However, the literature contains only a few studies on snakes. In serpents, species related to *Centrorhynchus* were reported in tiger keelback snakes (Choi *et al.*, 2010) and Indian cobras (Khan, Khatoun, & Bilquees, 2002). Therefore, Chinese ratsnakes were also an alternative host of *C. aluconi*, which may ecologically transmit from the snake's prey. However, *Centrorhynchus* of this finding is necessary to precisely identify as *C. aluconi* or related species. For the further study, the molecular methods such as DNA barcode of mitochondrial or nuclear genes may be preferred to the conformation of this species.

The surface topography of the hooks was not different to that reported in several studies on the surface of acanthocephalan hooks under low magnification (Amin, Heckmann, Zargar, Chishti, & El-Naggar, 2012; Gupta, Gupta & Singhal, 2015; Lunaschi & Drago, 2010). Under high magnification, the ribbed pattern on the surface of *C. cf. aluconi* was similar to that of *C. globirostris* (Amin, Heckmann, Wilson, Keele, & Khan, 2015), but the grooves on the hook surfaces of *C. cf. aluconi* were narrower than those of *C. globirostris*. To compare these properties with the related genera, Amin, Heckmann, and Bannai (2018) investigated the longitudinal grooves on the cortical hook surface of *Cavisoma magnum*. However, the longitudinal grooves on the hook surface of *C. cf. aluconi* were deeper than those of *C. magnum*. The hooks of *Cathayacanthus spinitruncatus* were dominated by a longitudinally ribbed surface (Amin, Heckmann, & Van Ha, 2014) differed from those of *C. cf. aluconi*. In addition, the hooks of *C. cf. aluconi* showed a longitudinally folded surface but were thinner and narrower than those of *Neoechinorhynchus dimorphospinus*, according to the study of Amin, Heckmann, Ali, El Naggar, and Khamees (2015). These results might increase our knowledge of the microstructure of *C. aluconi* and might yield important information to help identify this species. For the ultrastructure under TEM, the hook layers of *C. cf. aluconi* consisted of five layers. This result is different from the proboscis hooks of *Acanthocephalus lucii*, which were composed of three layers, and studies on the ultrastructure of *Centrorhynchus* and other species of acanthocephalan are limited (Brázová, Poddubnaya, Miss, & Hanzelová, 2014). The present study may be the first investigation on the ultrastructural makeup of the hooks of *C. aluconi*. In FESEM-EDX, the elemental intensity was different between the surface and the inside of the hooks of *C. cf. aluconi*. Only the signal intensity of F was detected in the core of the hooks. Recently, Ha, Amin, Ngo, and Heckmann (2018) reported an X-ray elemental scan of *Pararhadinorhynchus magnus* hooks, that showed the predominant elements to be composed of P and Ca; S and Mg were also detected. Furthermore, Brázová, Poddubnaya, Miss, and Hanzelová (2014) showed the detection



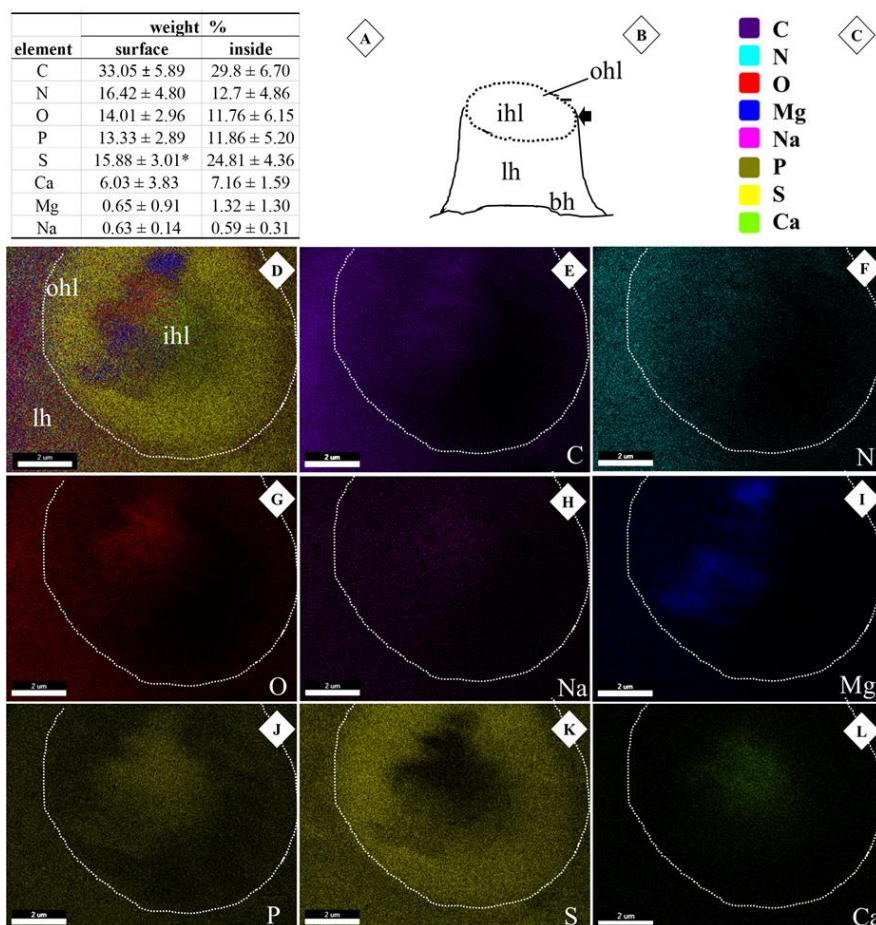


Figure 3. FESEM-EDX analyses of the hooks of *C. cf. aluconi*. Element data for the hook surface and the inside of the hook (A). Illustration of the transverse section of the hook for FESEM-EDX mapping (B). The arrow indicates the position of the cut. Color boxes indicate each element in FESEM-EDX mapping (C). Merged images for all elemental distributions (D). Distributions of carbon (E), nitrogen (F), oxygen (G), sodium (H), magnesium (I), phosphorus (J), sulfur (K), and calcium (L). Base of the hook (bh), inner hook layer (ihl), outer hook layer (ohl), and lateral surface of the hook (lh).

of Ca, Mg, P, and S from the hooks of *A. lucii*. Therefore, the P, S, and Ca of *C. cf. aluconi* hooks associated with the chemical profiles of *P. magnus* and *A. lucii* may be the main elemental composition of hooks in acanthocephalans. High intensities of C, O, and N in hooks of *C. cf. aluconi* were also confirmed in a study on *P. magnus* by Ha, Amin, Ngo, and Heckmann (2018). C, O, and N are simple elements of living cells (Alberts *et al.*, 2002). However, the findings of high S in the hooks of this study suggested that S might be a specific element deposition in the hooks of *C. cf. aluconi*. Although S is well known to be an important element for living organisms, including lower invertebrates (Grühlke & Slusarenko, 2012), it is novel to suggest the biochemical or physiological processes of S in the hooks of *C. cf. aluconi*.

To our present knowledge, the data on the micro- and ultrastructure of hooks might support the identification of *C. aluconi*. Element profiles of the hooks had a different distribution between the surface and the inside.

## References

- Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). *Molecular biology of the cell* (4<sup>th</sup> ed.). New York, NY: Garland Science.
- Amin, O. M. (2013). Classification of the acanthocephala. *Folia Parasitologica (Praha)*, 60(4), 273-305. doi: 10.14411/fp.2013.031
- Amin, O. M., Crompton, D. W. T., & Brent, B. N. (1985). Classification. In D.W.T. Crompton & B.B. Nickol (Eds.), *Biology of the Acanthocephala* (pp. 27-72). London, England: Cambridge University Press.
- Amin, O. M., Heckmann, R. A., Ali, A. H., El Naggar, A. M., & Khamees, N. R. (2015). New features of *Neoechinorhynchus (Neoechinorhynchus) dimorphospinus* (Acanthocephala: Neoechinorhynchidae) from recent collections in the Arabian Gulf using SEM, with notes on histopathology. *Comparative Parasitology*, 82(1), 60-67. doi:10.1654/4717.1

- Amin, O. M., Heckmann, R. A., & Bannai, M. A. (2018). *Caviso-ma magnum* (Cavisomidae), a unique Pacific acanthocephalan redescribed from an unusual host, *Mugil cephalus* (Mugilidae), in the Arabian Gulf, with notes on histopathology and metal analysis. *Parasite*, 25, 5. doi:10.1051/parasite/2018006
- Amin, O. M., Heckmann, R. A., & Van Ha, N. (2014). Acanthocephalans from fishes and amphibians in Vietnam, with descriptions of five new species. *Parasite*, 21, 53. doi:10.1051/parasite/2014052
- Amin, O. M., Heckmann, R. A., Wilson, E., Keele, B., & Khan, A. (2015). The description of *Centrorhynchus globirostris* n. sp. (Acanthocephala: Centrorhynchidae) from the pheasant crow, *Centropus sinensis* (Stephens) in Pakistan, with gene sequence analysis and emendation of the family diagnosis. *Parasitology Research*, 114(6), 2291-2299. doi:10.1007/s00436-015-4424-0
- Amin, O. M., Heckmann, R. A., Zargar, U. R., Chishti, M. Z., & El-Naggar, A. M. (2012). The morphology of the long forgotten *Pomphorhynchus kashmirensis* (Acanthocephala: Pomphorhynchidae) from freshwater fish in Kashmir using SEM, with notes on histopathology. *Scientia Parasitologica*, 13(3), 93-99.
- Baker, M. K., Lange, L., Verster, A., & van der Plaats, S. (1989). A survey of helminths in domestic cats in the Pretoria area of Transvaal, Republic of South Africa. Part 1: The prevalence and comparison of burdens of helminths in adult and juvenile cats. *Journal of the South African Veterinary Association*, 60(3), 139-142.
- Brázová, T., Poddubnaya, L. G., Miss, N. R., & Hanzelová, V. (2014). Ultrastructure and chemical composition of the proboscis hooks of *Acanthocephalus lucii* (Müller, 1776) (Acanthocephala: Palaeacanthocephala) using X-ray elemental analysis. *Folia parasitologica*, 61(6), 549-557. doi:10.14411/fp.2014.053
- Choi, C. J., Lee, H. J., Go, J. H., Park, Y. K., Chai, J. Y., & Seo, M. (2010). Extraintestinal migration of *Centrorhynchus* sp. (Acanthocephala: Centrorhynchidae) in experimentally infected rats. *Korean Journal of Parasitology*, 48(2), 139-143. doi:10.3347/kjp.2010.48.2.139
- Dimitrova, Z. M., & Gibson, D. I. (2005). Some species of *Centrorhynchus* Lühe, 1911 (Acanthocephala: Centrorhynchidae) from the Collection of the Natural History Museum, London. *Systematic Parasitology*, 62, 117-134. doi:10.1007/s11230-005-5486-7
- Ewald, J. A., & Crompton, D. W. (1993). *Centrorhynchus aluconis* (Acanthocephala) and other helminth species in tawny owls (*Strix aluco*) in Great Britain. *Journal of Parasitology*, 79(6), 952-954.
- Goldberg, S. R., Bursey, C. R., & Cheam, H. (1998). Helminths of six species of Anolis lizards (Polychrotidae) from Hispaniola, West Indies. *Journal of Parasitology*, 84(6), 1291-1295.
- Gruhlke, M. C., & Slusarenko, A. J. (2012). The biology of reactive sulfur species (RSS). *Plant Physiology and Biochemistry*, 59, 98-107. doi:10.1016/j.plaphy.2012.03.016
- Gupta, N., Gupta, K., & Singhal, P. (2015). Description of *Pallisentis (Brevitritospinus) punctati* n. sp. (Acanthocephala: Quadrigyridae) from *Channa punctatus* in Bareilly, Uttar Pradesh, India. *Iranian Journal of Parasitology*, 10, 605-616.
- Ha, V. N., Amin, O. M., Ngo, H. D., & Heckmann, R. A. (2018). Descriptions of acanthocephalans, *Cathayacanthus spinitruncatus* (Rhadinorhynchidae) male and *Pararhadinorhynchus magnus* n. sp. (Diplosentidae), from marine fish of Vietnam, with notes on *Heterosentis holospinus* (Arhythmacanthidae). *Parasite*, 25, 35. doi:10.1051/parasite/2018 032
- Halajian, A., Smales, L. R., Tavakol, S., Smit, N. J., & Luus-Powell, W. J. (2018). Checklist of Acanthocephalan parasites of South Africa. *ZooKeys*, 789, 1-18. doi:10.3897/zookeys.789.27710
- Khan, A., Khatoon, N., & Bilqees, F. M. (2002). *Centrorhynchus sindhensis*, new species (Acanthocephala: Centrorhynchinae) from the snake (*Naja naja*) intestine. *Pakistan Journal of Zoology*, 34, 309-310.
- Komorová, P., Špakulová, M., Hurníková, Z., & Uhrín, M. (2015). Acanthocephalans of the genus *Centrorhynchus* (Palaeacanthocephala: Centrorhynchidae) of birds of prey (Falconiformes) and owls (Strigiformes) in Slovakia. *Parasitology Research*, 114(6), 2273-2278. doi:10.1007/s00436-015-4420-4
- Lunaschi, L., & Drago, F. (2010). A new species of *Centrorhynchus* (Acanthocephala, Centrorhynchidae) endoparasite of *Guirra guirra* (Aves, Cuculidae) from Argentina. *Helminthologia*, 47, 38-47. doi:10.2478/s11687-010-0007-x
- McDonald, M. E. (1988). Key to acanthocephala reported in waterfowl. United State Department of the Interior, Fish and Wildlife Service. Retrieved from <https://apps.dtic.mil/dtic/tr/fulltext/u2/a322632.pdf>
- Richardson, D. J., Smales, L. R., Ghorbani, M. N., & Halajian, A. (2017). *Centrorhynchus* sp. (Acanthocephala: Centrorhynchidae) from Stray Dogs (*Canis familiaris*) in Qom Iran. *Comparative Parasitology*, 84(2), 159-162. doi:10.1654/1525-2647-84.2.159
- Santos, V. G. T., & Amato, S. B. (2010). *Rhinella fernandezae* (Anura, Bufonidae) a paratenic host of *Centrorhynchus* sp. (Acanthocephala, Centrorhynchidae) in Brazil. *Revista mexicana de biodiversidad*, 81, 53-55.
- Smales, L. R. (2007). Acanthocephala in amphibians (Anura) and reptiles (Squamata) from Brazil and Paraguay with description of a new species. *Journal of Parasitology*, 93(2), 392-398.
- Torres, P., & Puga S. (1996). Occurrence of cystacanths of *Centrorhynchus* sp. (Acanthocephala: Centrorhynchidae) in toads of the genus *Eupsophus* in Chile. *Memórias do Instituto Oswaldo Cruz*, 91(6), 717-719. doi:10.1590/S0074-02761996000600011