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Research note

Chemical Analysis of Metals in Acanthocephalans Using Energy Dispersive X-Ray Analysis (EDXA) in Conjunction with a Scanning Electron Microscope (SEM)

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ABSTRACT: Using energy-dispersive x-ray analysis (EDXA) in conjunction with a scanning electron microscope, high levels of sulfur were recorded in proboscis hooks of 5 species of Acanthocephala: *Neoechinorhynchus qatarensis* Amin, Saoud, and Alkuwari, 2002; *Acanthocephalus dirus* (Van cleave, 1931); *Neoechinorhynchus idahoensis* Amin and Heckmann, 1992, *Echinorhynchus salmonis* (Müller, 1784); and *Pseudoacanthocephalus* sp. Petrochenko, 1956. Sulfur was also present in eggs of *N. qatarensis*. No sulfur was recorded in the body of worms. The 5 species of Acanthocephala were obtained from several fish hosts and 1 toad species from 7 geographical locations. Sulfur ions are probably present in disulphide bonds characteristic of 2 amino acids, cystine and cysteine, which are common amino acids in the protein of mammalian hair and horns and are probably associated with the hardness of acanthocephalan proboscis hooks and egg shells.

KEY WORDS: Acanthocephala, metal analysis, EDXA, sulfur in proboscis hooks and eggs.

A prominent structure in all acanthocephalans is the armed proboscis. Proboscis hooks and eggs are of great survival significance and are of taxonomic importance for species identification. There is a paucity of data on the chemical composition of hooks and eggs. During a routine examination with energy-dispersive x-ray analysis (EDXA) of *Neoechinorhynchus qatarensis* Amin, Saoud, and Alkuwari, 2002, it was noted that the hooks and eggs had a high sulfur content compared with the body of the organism. Four additional species of Acanthocephala were examined from at least 5 fish hosts and from the common Sunda toad representing 6 additional geographical locations to validate the initial observation.

During the past 3 decades, x-ray (EDXA) analysis has been developed and used in conjunction with electron optics in this Brigham Young University (BYU) laboratory and others (Lee, 1992; Heinrich, 1981). This technique can detect and locate chemical elements in small defined areas, and it can be both a quantitative and qualitative technique (Heckmann, 1996).

Energy-dispersive x-ray analysis is practically a nondestructive method for the microanalysis of element composition. Requirements for sample preparation are minimal, and very small samples can be analyzed. Energy-dispersive x-ray analysis is a technique in which x-ray emissions from the sample are sorted by energy rather than by wavelength using a diffraction crystal. When electrons of appropriate energy impinge on a sample, they cause the excitation of x-rays whose energies and relative abundance depend upon the composition of the sample. This technique enables the scientist to detect, measure, and determine the location of chemical elements within living and nonliving samples. Compared with wavelength dispersive analysis, this technique can detect nearly all chemical elements in a sample during a single run rather than only 1 element per run (Vaughn, 1989; Whallon et al., 1989; Johnson, 1993). The objective of our study was to use EDXA on different species of Acanthocephala to detect unique concentrations of chemical elements.

Specimens of 5 species of Acanthocephala (*Acanthocephalus dirus* (Van Cleave, 1931); *Echinorhynchus salmonis* Müller, 1784; *Neoechinorhynchus idahoensis* Amin and Heckmann, 1992; *N. qatarensis* Amin, Saoud, and Alkuwari, 2002; and *Pseudoacanthocephalus* sp. Petrochenko, 1956) were obtained from various hosts and locations as listed in Table 1. Each specimen, previously fixed in formalin, was dehydrated in an ascending series of ethanol solutions to 70% and then stored until needed. Standard methods were used to prepare each specimen for SEM analysis (Lee, 1993), which included critical point drying and attaching the specimen to stubs. Samples attached to stubs were examined with an Environmental SEM (XL3OESEMFEG) equipped with a Phoenix energy-dispersive x-ray analyzer. Each specimen was analyzed twice with both the

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Table 1. Hosts and locations for observed Acanthocephala.

Host	Species of acanthocephala (abbreviation)	Host location
Blue-barred parrot fish *Scarus ghobban* (Forsskål)	*Neoechinorhynchus qatarensis* (NQ)	Arabian Gulf: Qatar waters
Various Hosts	*Acanthocephalus dirus* (AAD) (BAD)	Pike River, Wisconsin
Brook trout *Salvelinus fontinalis* (Mitchill)	*Acanthocephalus dirus* (BAD)	Various rivers, New Hampshire
White Sucker *Catostomus commersoni* (Lacépède)	*Acanthocephalus dirus* (BAD)	Various rivers, New Hampshire
Bridgelip Sucker *Catostomus columbianus* (Eigemann and Eigemann)	*Neoechinorhynchus idahoensis* (CNI)	Salmon River, Idaho
Coho Salmon *Oncorhynchus kisutch* (Walbaum)	*Echinorhynchus salmonis* (DES)	Lake Michigan, Wisconsin
Chinook Salmon *Oncorhynchus tsawytscha* (Walbaum)	*Echinorhynchus salmonis* (DES)	Lake Michigan, Michigan
Sunda Toad *Bufo melanostictus* (Schneider)	*Psuedoacanthocephalus* sp. (PAS)	Tam Dao National Park, Vinh Phuc Province, Vietnam

body and hooks activated with x-ray. Eggs of *N. qatarensis* were processed by allowing a smear of eggs to dry on a glass coverslip and then attaching the coverslip to a SEM specimen stub. A glass coverslip without eggs was evaluated as a standard.

Results of 60-sec scans are depicted in Figures 1–3. A high level of sulfur was present in the proboscis hooks, but not the body, of all 5 species of Acanthocephala examined (Table 2). Eggs and embryos of *N. qatarensis* were also scanned to confirm a report (Anantaraman, 1996), using a different technique, of sulfur detection in eggs (Fig. 3, Table 3).

X-ray microanalysis, in conjunction with SEM and transmission electron microscopy (TEM), is a valuable tool in understanding and evaluating levels of chemical elements in tissue (Vaughn, 1989; Heckmann, 1996). One concern about x-ray microanalysis has been the quantitative application of the technique. This is one reason why we have based our results on the peak height after a 60-sec run for each specimen. The technique is excellent for readily locating minute amounts of chemical elements in fish tissue (Heckmann, 1996). Sures (2003) reviewed work on the detection of heavy metals in fish parasites using electrothermal atomic absorption spectrometry. We have had success tracing heavy metals in fish and fish parasites using x-ray microanalysis.

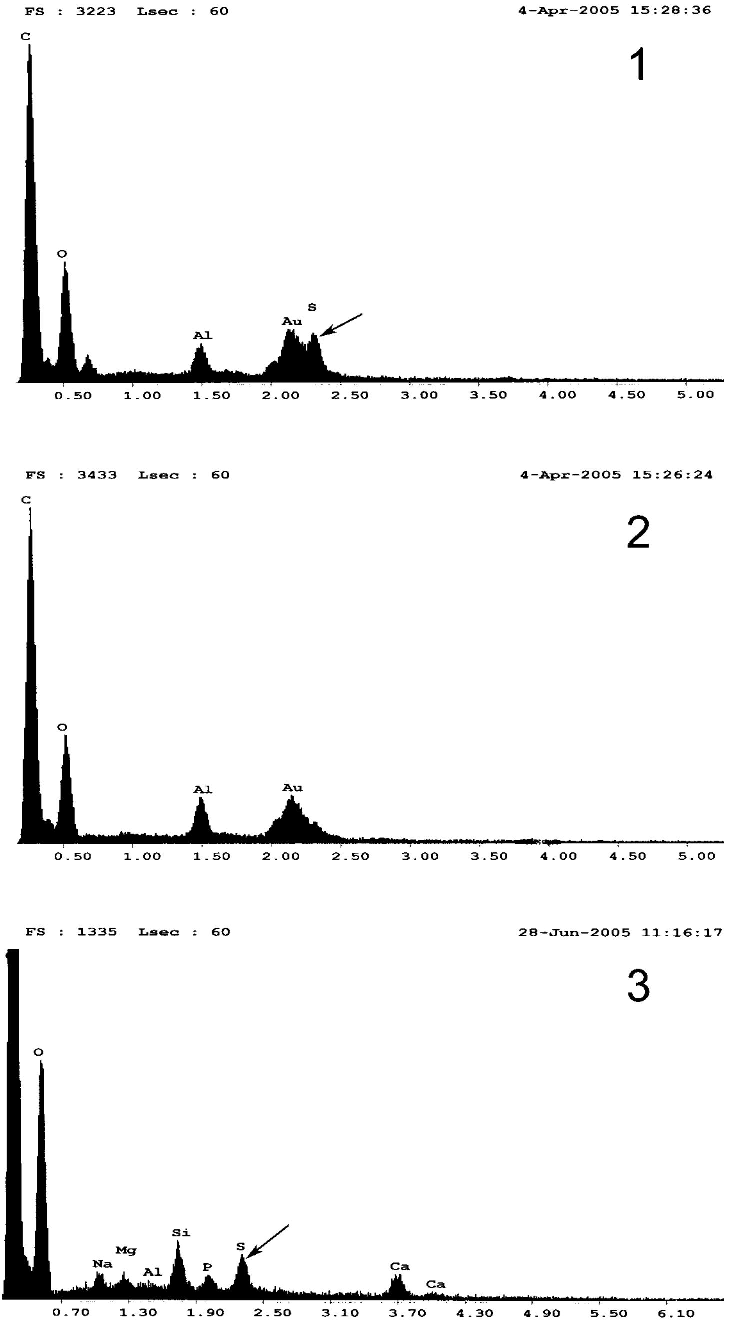
Proboscis hooks and eggs exhibited high levels of sulfur not observed in the rest of the organism. Anantaraman (1976) noted sulfur in the outer layers of eggs of the acanthocephalan *Acanthosentis* sp. using histochemical techniques. It was postulated that the sulfur ions are present in disulfide bonds characteristic of amino acids such as cystine and cysteine, which are common in keratin in mammalian horns (Stedman, 2001). The relationship between sulfur in cystine and cysteine and the hardness of

Table 2. Peak heights (mm) after a 60-sec run with x-ray microanalysis (EDXA).

Chemical elements detected*	Specimen** AAD		BAD		CNI		DES		NQ		PAS	
	Hook	Body	Hook	Body	Hook	Body	Hook	Body	Hook	Body	Hook	Body
Aluminum	13	12	0	0	0	0	0	0	0	0	0	0
Gold	14	13	16	8	9	7	25	8	30	16	13	9
Sulfur	15	4	15	0	11	0	14	0	20	0	11	0

* Specimens are coated with gold. Specimen holders (stubs) contain aluminum. Elements (C, H, N, Ca) common in protoplasm for Acanthocephala are omitted (Fig. 1).

** AAD, *Acanthocephalus dirus* from various hosts in Wisconsin; BAD, *A. dirus* from white sucker and brook trout in New Hampshire; CNI, *Neoechinorhychus idahoensis* from bridgelip sucker in Idaho; DES, *Echinorhynchus salmonis* from Coho and Chinook salmon in Lake Michigan; NQ, *Neoechinorhynchus qatarensis* from Parrot fish in the Persian Gulf. PAS, *Pseudoacanthocephalus* sp. from Sunda Toad in Tam Dao National Park (Vietnam).



Figures 1–3. Typical charts recording elemental composition using energy-dispersive x-ray analysis (EDXA) in conjunction with a scanning electron microscope (SEM). **1.** Hooks of *Acanthocephalus dirus*, peaks for observed chemical elements after a 60-sec run include a sulfur peak (arrow). **2.** Body of *Acanthocephalus dirus*, peaks for observed chemical elements after a 60-sec run lack a sulfur peak. **3.** Eggs of *Neoechinorhynchus qatarensis*, peaks for observed chemical elements after a 60-sec run include a sulfur peak (arrow).

Table 3. Peak heights (mm) after a 60-sec run with energy-dispersive x-ray microanalysis (EDXA) in eggs and embryos of *Neoechinorhynchus qatarensis*.

Chemical element detected	*Neoechinorhynchus qatarensis*		Glass slide (standard)
	Egg shell	Egg embryo	
Sodium	10	6	21
Aluminum	6	4	3
Magnesium	8	5	8
Silicon	21	17	89
Potassium	0	0	8
Sulfur	15	13	2
Calcium	8	8	2
Phosphorus	7	8	1

such mammalian structures suggests that acanthocephalans use the same system to provide hardness to proboscis hooks and egg shells for the obvious survival advantages of efficient attachment and reproduction.

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