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ORIGINAL RESEARCH ARTICLE

Electron Optics Study (SEM, EDXA) of *Diplozoon paradoxum* (Nordman, 1832) (Diplozoidae, Trematoda) from the common carp, *Cyprinus carpio* L. (Cyprinadae, Osteoichthyes) in Vietnam with comments on potential host fish

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Abstract. Specimens of *Diplozoon paradoxum* Nordman 1832 were scanned with both SEM (Scanning Electron Microscopy) and EDXA (Energy Dispersive Analysis for X-ray). The X shaped, fused individuals were well displayed with the prominent prohaptor and opisthohaptor both containing host attachment structures. *D. paradoxum* has a wide range of fish hosts. These specimens come from the common carp, *Cyprinus carpio* L. in Vietnam. The paired prohaptor is divided by a partition which separates the muscular oral sucker. There are numerous muscular convolutions within the sucker. The body is covered with spines and convolutions. The opisthohaptor contains four pair of laterally positioned corrugated clamps and a rectangular shaped concave terminal end. Eggs are oval and numerous. Scans for elemental analysis for the body regions and eggs are included. This is the first published study using electron optics for *D. paradoxum.*

Keywords: *Diplozoon paradoxum;* Monogenea; Electron Optics; SEM; EDXA.

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Introduction

The genus *Diplozoon* Nordman 1832 is characterized by an opisthohaptor rectangular in shape with 4 pairs of clamps and a pair of inconspicuous posterior anchors. As a member of the Diplozooidae Tripathi, 1959 family and the Diplozooidea superfamily features include two adults which are permanently fused in the form of letter X. Prohaptor consists of paired suckers opening into the oral cavity. Genital pore is in the posterior half of the body. The genital pore of the paired adults are united with complimentary pores for each partner worm. Due to the fusion of the 2 bodies it is considered a 2-headed parasite.

Further description of the *Diplozoon* include: 4 pairs of lateral clamps for the terminal opisthohaptor. The opisthohaptor is terminal, rectangular in shape and concave ventrically. Eggs are large with a very long coiled filament at the opercular pole. Occasionally the polar filament is absent.

Diplozoon paradoxum is a Monogenoidea found in freshwater fishes in Asia and Europe that is known to have complete monogamy. This parasite is commonly found on the gills of European cyprinid fishes. Many fish host genera are listed by Yamaguti (1963): *Cyprinus, Esox, Abramis, Barbus, Carassius, Rutilus, Cottus, Amar, Acipenser, Parabramis, Silurus* etc. Its size is usually around 0.7 cm (approximately the size of a fingernail). It is a platyhelminth and has several hooks at its mouth which it will use to grab on the gills of a fish. From there it will feed on the blood of a Cyprinid or other fish host. Cyprinids are smaller fish that include the goldfish. *Diplozoon paradoxum* have bilateral symmetry and exhibit strong seasonal variation in their reproductive activity. Unlike most parasites that produce gametes all year, *paradoxum* gametes are produced primarily during the spring, with the highest production from May to June and continuing through the rest of the summer. The eggs of the *Diplozoon paradoxum* are laid in a fresh water fish's gills. There it hatches into a larva stage (diporpa). It will remain in that stage unless two larvae come together. Then the two larvae will undergo metamorphosis and become fused together (Kagel and Taraschewski, 1993).

The life cycle of the *D. paradoxum* is unique. A diporpa juvenile can live for several months, but it cannot develop further until encountering another diporpa; unless this happens, the diporpa usually will die. When one diporpa finds another, each attaches its sucker to the dorsal papilla of the other. This begins one of the most intimate associations of two individuals of the animal kingdom. The two worms fuse completely, with no trace of partitions separating them. The fusion stimulates maturation. Gonads appear; the male genital duct of one terminates near the female genital duct of the other, permitting cross-fertilization. Two more pairs of clamps develop in the opisthaptor of each. Adults apparently can live in this state for several years.

Classification

Phylum:	Platyhelminthes
Class:	Trematoda
Subclass:	Monogenea
Order:	Polyopisthocotylea
Family:	Diplozoidae
Genus:	*Diplozoon*
Species:	*D. paradoxum*

Binomial name
Diplozoon paradoxum (Nordmann 1832)
Host Fish
German Carp: *Cyprinus carpio*

Cyprinus carpio known as the common carp has a worldwide distribution and has been a major species for aquaculture as a food fish.

Carp are various species of oily freshwater fish of the family Cyprinidae, a very large group of fish native to Europe and Asia. The cypriniformes (family Cyprinidae) are traditionally grouped with the Characiformes, Siluriformes and Gymnotiformes to create the superorder Ostariophysi, since these groups have certain common features, such as being found predominantly in fresh water and that they possess Weberian ossicles (an anatomical structure originally made up of small pieces of bone formed from four or five of the first vertebrae); the most anterior bony pair is in contact with the extension of the labyrinth and the posterior with the swimbladder. The function is poorly understood, but this structure is presumed to take part in the transmission of vibrations from the swimbladder to the labyrinth and in the perception of sound, which would explain why the Ostariophysi have such a great capacity for hearing (Varadi, 2001).

Carp have long been important food fish to humans, as well as popular ornamental fishes such as the various goldfish breeds and the domesticated common carp variety known as koi. As a result, carp have been introduced to various locations, though with mixed results. Several species of carp are listed as invasive species by the U.S. Department of Agriculture, and worldwide large sums of money are spent on carp control (Varadi, 2001; Billard, 1995).

Various species of carp have been domesticated and reared as food fish across Europe and Asia for thousands of years. These various species appear to have been domesticated independently, as the various domesticated carp species are native to different parts of Eurasia. For example, the common carp, *Cyprinus carpio*, is originally from Central Europe. Several carp species (collectively known as Asian carp) were domesticated in East Asia. Carp that are originally from South Asia, for example catla *(Gibelion catla),* rohu *(Labeo rohita)* and mrigal *(Cirrhinus cirrhosus),* are known as Indian carp. Their hardiness and adaptability have allowed domesticated species to be propagated all around the world (Böhme, 2004).

Although the carp was an important aquatic food item, as more fish species have become readily available for the table, the importance of carp culture in Western Europe has become less important. Demand has decline, partly due to the appearance of more desirable table fish such as trout and salmon through intensive farming, and environmental constraints. However, fish production in ponds is still a major form of aquaculture in Central and Eastern Europe, including the Russian Federation, where most of the production comes from low or intermediate-intensity ponds. In Asia, the farming of carp continues to surpass the total amount of farmed fish volume of intensively sea-farmed species, such as salmon and tuna (Chistiakov and Voronova, 2009).

Another potential host for *Diplozoon paradoxum* in Vietnam is the snakehead fish. This is an excellent food fish in most countries. The snakeheads are members of the freshwater perciform fish family Channidae, native to Africa and Asia. These elongated predatory fish are distinguished by a long dorsal fin, large mouth and shiny teeth. They breathe air with a suprabranchial organ, a primitive from of a labyrinth organ. The two extant genera are *Channa* in Asia and *Parachanna* in Africa, consisting of 30-35 species.

Snakeheads can become invasive species and cause ecological damage because they are top-level predators, meaning they have no natural enemies outside of their native environment. Not only can they breath atmospheric air, but they can also survive on land for up to four days, provided they are wet, and are known to migrate up to ¼ mile on wet land to other bodies of water by wriggling their body and fins. Each spawning-age female can release up to 15,000 eggs at once. Snakeheads can mate as often as five times a year. This means in just two years, a single female can release up to 150,000 eggs (Courtenay and Williams, 2004).

Snakeheads are thrust-feeders which consume plankton, aquatic insects, and mollusks when small. As adults, they mostly feed on other fish, such as carp, or on frogs. In rare cases, small mammals. Snakeheads became a national news topic in the US because of the appearance of northern snakeheads spawning in a Crofton, Maryland pond in 2002. Northern snakeheads became permanently established in the Potomac River around 2004, and possibly established in Florida. Apparently established specimens have been found in Wawayanda, New York, two ponds in Philadelphia, Pennsylvania and reservoirs in Northern Carolina (Courtenay and Williams, 2004; Britz, 2007).

From 2002 to 2003, one Los Angeles supermarket was found to have illegally sold approximately $25,000 worth of live snakeheads, which caused breakouts in local ecosystems.

In what determined by the Army Corps of Engineers to be an isolated incident, a fisherman caught a single snakehead on October, 2004 while fishing form Lake Michigan at Burnham Harbor in Chicago, Illinois. According to the Unived States Environmental Protection Agency, snakeheads have also been spotted in California, Florida, Hawaii, Maine, Maryland, Massachusetts, Arkansas and Rhode Island (Zambito, 2011).

On April 25, 2011, a northern snakehead was found above Virginia's Great Falls near Whites Ferry. Great Falls was supposedly a natural barrier that the fish was unable to cross. It is apparently the first time a northern snakehead was found above the falls (Zambito, 2011).

Why so much press just for a fish? The Northern Snakehead, *Channa argus,* is no ordinary fish, biologists explain. It is a voracious top-level predator, meaning that it has no natural enemies, and could decimate populations of native fish. About 90% of its diet consists of other fish, though it also eats crustaceans, insects, and plants. In its native range it can live in water with temperatures ranging from 0 to 30 degrees C; it is found in muddy or vegetated ponds, swamps, and slow moving streams. Snakeheads can breathe air and survive for up to four days out of water, and can survive for longer periods of time when burrowed in the mud. They are capable of traveling over land to new bodies of water by wriggling their bodies over the ground. These features are adaptations to the seasonal drying of shallow bodies of water in the snakehead's native habitat in China and allow it to disperse widely should local conditions become unfavorable. It is capable of surviving in much of North America should it become established. An established population of snakeheads in Maryland could have long-term disastrous consequences for the ecology of the region (Courtenay and Williams, 2004; Zambito, 2011).

Materials and methods

Specimens of *Diplozoon paradoxum* were provided by the second author from common carp, *Cyprinus carpio* in Vietnam. There are many potential hosts for *Diplozoon paradoxum* in Vietnam. The specimens were fixed in 70% ethyl alcohol (ETOH). Selected specimens were placed in critical point drying (CPD) baskets then dehydrated using an ETOH series of 95% and 100% for at least 10 minutes per soak followed by critical point drying (Lee, 1992). Samples were mounted on SEM sample mounts (stubs), gold coated and observed with a scanning electron microscope (XL30ESEMFEG; FEI, Hillsboro, Oregon). Digital images of the specimen were obtained using digital imaging software attached to a computer.

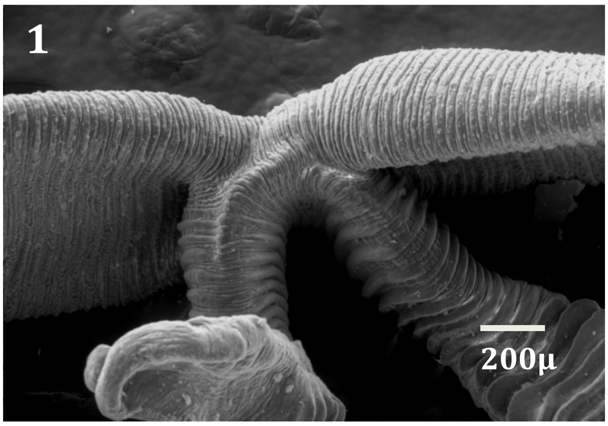
For X-ray microanalysis, standard methods of SEM preparation were used (Lee, 1992). Coated specimens were examined with an Environmental SEM (XL30ESEMFEG) equipped with a Phoenix Energy Dispersive X-ray Analyzer. X-ray spot analysis was performed at 15 KV with a spot size of 5 and results were represented in charts and recorded with digital imaging software attached to the computer (Lee, 1992).

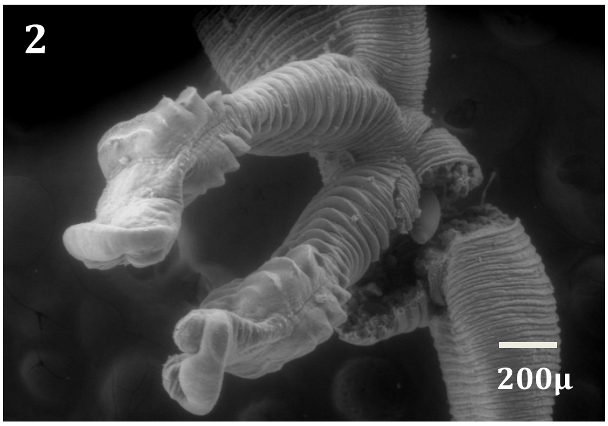
Results

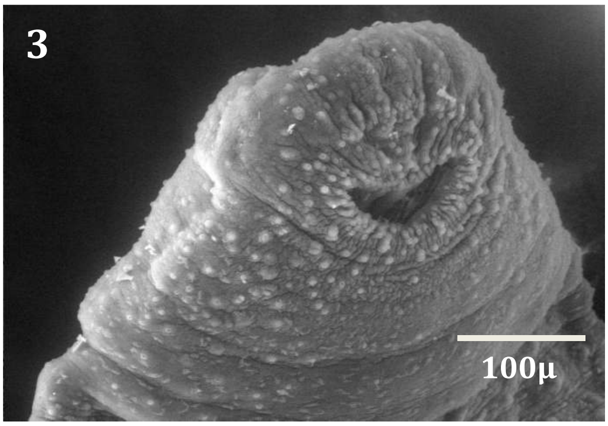
Figures 1 to 12 represent the results of the scans completed for *Diplozoon paradoxum* using scanning electron microscopy (SEM). Figure 1 depicts the characteristic shape of the 2 fused monogeneans. The central part of each organism is fused together (figure 2) forming the characteristic X shape of *D. paradoxum*. The body is covered with knob-like projections which are porous (micropores). There are numerous annulations along the body of the worms. Both the prohaptor and opisthohaptor are prominent.

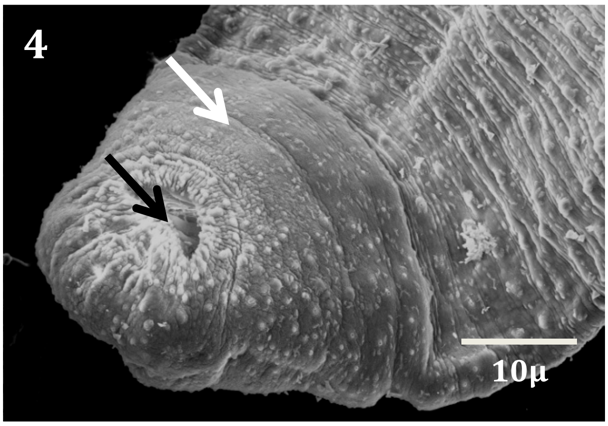
The prohaptor is represented by figures 3, 4, and 5. The oral sucker of the prohaptor is prominent with a definite partition between the two parts giving it a paired appearance. These openings yield entrance into the oral cavity and the blind digestive system. The partition dividing the oral sucker is prominent for figure 4. Note the numerous convolutions in the oral region which will aid in attachment. The opisthohaptor is rectangular in shape with a concave terminal shape. There are 4 pairs of lateral corrugated "brushes" or clamps which aid in host attachment (figures 6, 7, 8, and 9). For the opisthohaptor note the attachment structures used by the ectoparasite (sucker, clamps and hooks). The body is covered with spines or tubercles from anterior to posterior (figures 10 and 11). There are numerous knobs (tubercles) surrounding the anterior region (figures 10 and 11). This monogenean is highly modified for a parasitic form of symbiosis. Figure 12 represents the eggs of *D. paradoxum*.

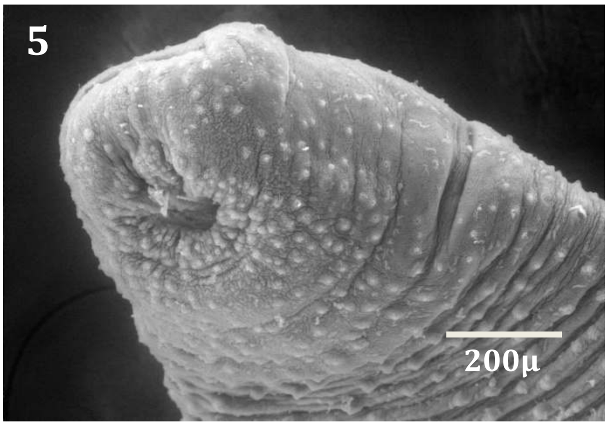
Figures 13, 14 and 15 are the elemental analysis scans of the parasite. Figure 14 is the anterior region near the prohaptor, while Figure 15 is the opisthohaptor region. Figure 13 is the scan of the egg.











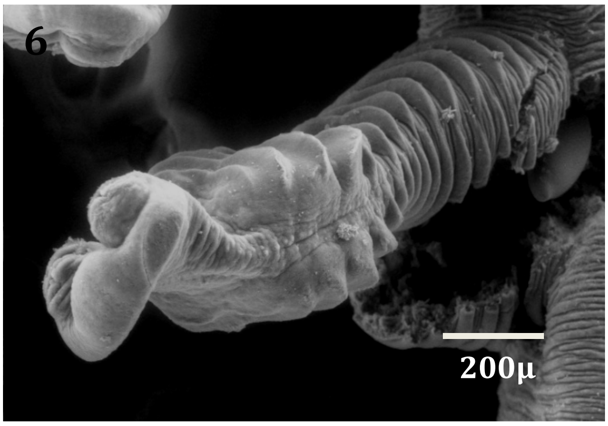
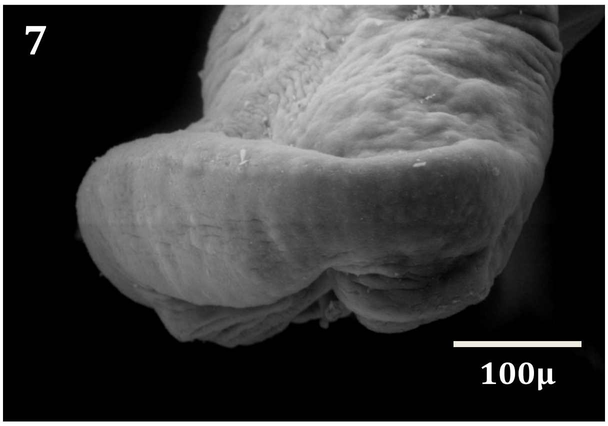
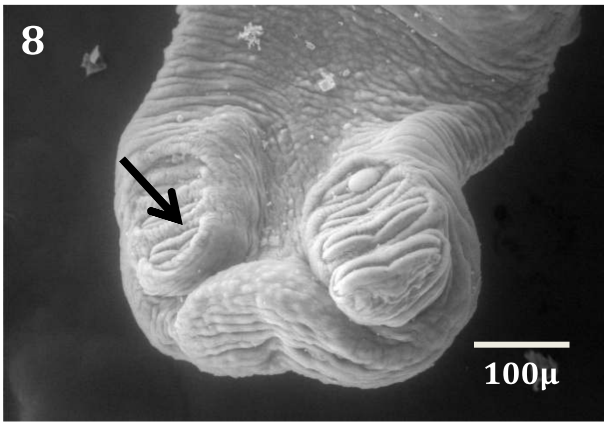
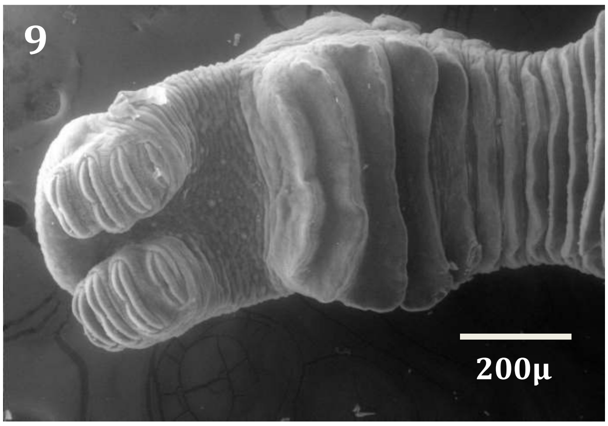
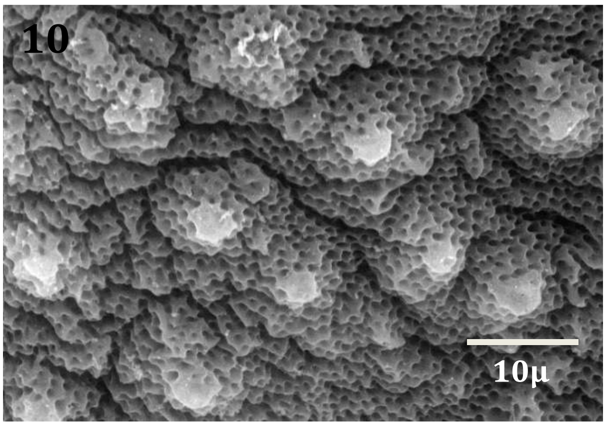


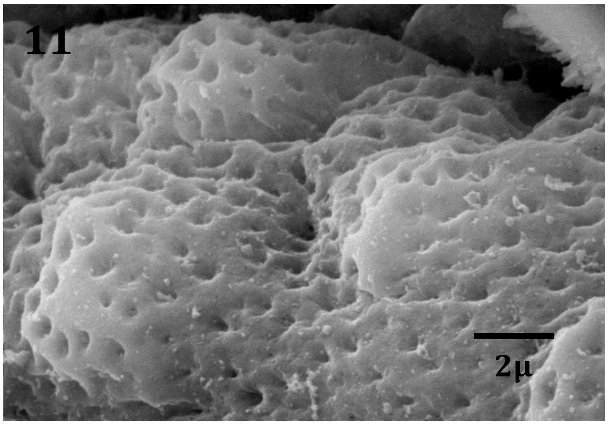
Figure 1. Two fused *Diplozoon paradoxum* showing the area where the two individuals fused together. Represents the fusion of two diporpa juveniles. **Figure 2.** Similar to figure 1 but the broken area shows how permanent the fusion is for the two individuals. Note the two foot-like opisthohaptors with the comb-like areas and the convoluted knobed body. **Figure 3.** The anterior end of *D. paradoxum* showing the oral cavity with the paired suckers for the prohaptor. Note the knob-like extensions (spines) on the surface of the body attached to the integument. **Figure 4.** An “en face” view showing the partitions (arrows) between the paired oral sucker. Numerous knobs or bumps on the surface. **Figure 5.** Frontal view of the prohaptor. Note the convoluted muscular rows around the oral cavity. Numerous knobs with openings or sensory pores.
Figure 6. The terminal part of the opisthohaptor with the concave end and the comb-like brushes on the lateral side.











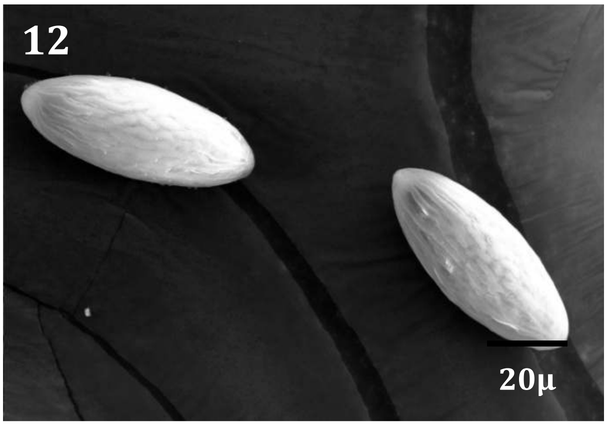
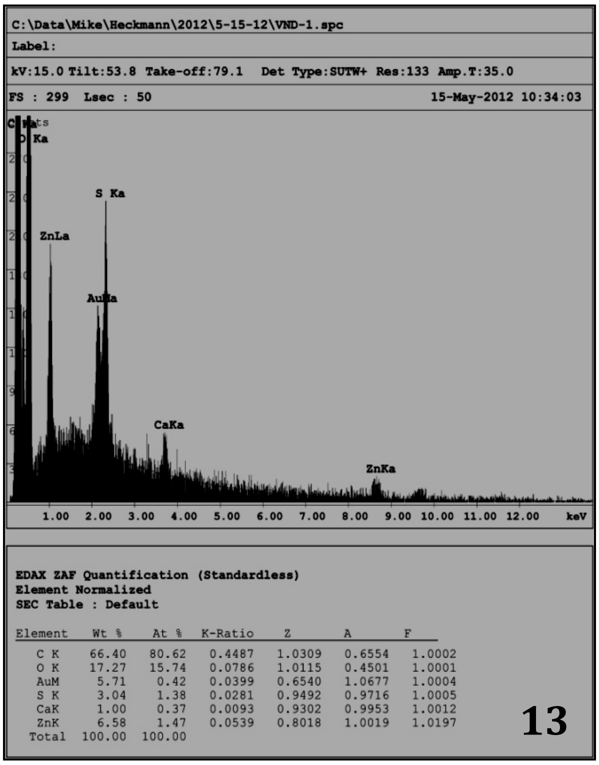


Figure 7. Higher magnification of Figure 6 depicting the concave terminal end for the posterior part of *D. paradoxum.* **Figure 8.** The brush-like combs for the opisthohaptor region of the ectoparasite used for host attachment. Again the corrugated body is visible. **Figure 9.** A contracted opisthohaptor showing the concave terminalis and the paired 4 rows of combs. **Figure 10.** Integument for *D. paradoxum* showing numerous bumps and micropores. The latter used for feeding. **Figure 11.** Higher magnification of Figure 10. **Figure 12.** Eggs for *D. paradoxum,* oval shape and numerous.



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   AuM     5.71    0.42   0.0399   0.6540  1.0677  1.0004
   S K     3.04    1.38   0.0281   0.9492  0.9716  1.0005
   CaK     1.00    0.37   0.0093   0.9302  0.9953  1.0012
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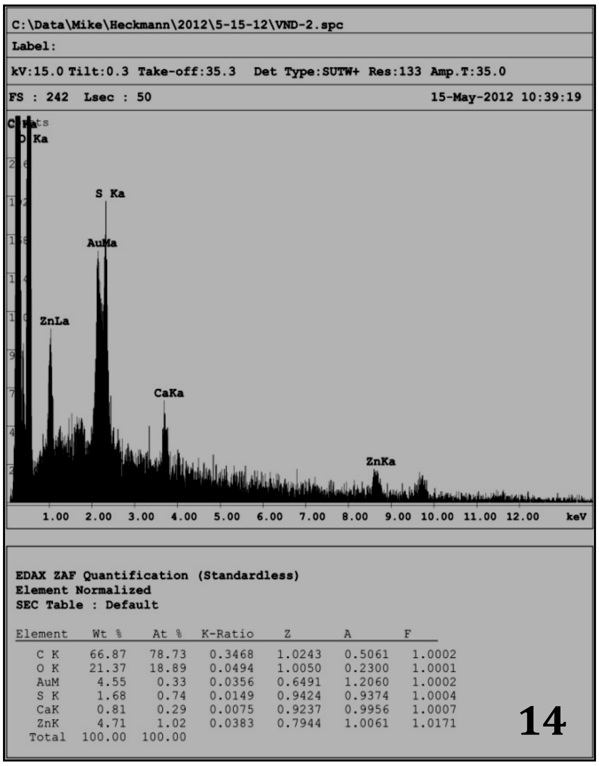
13



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   AuM     4.55    0.33   0.0356   0.6491  1.2060  1.0002
   S K     1.68    0.74   0.0149   0.9424  0.9374  1.0004
   CaK     0.81    0.29   0.0075   0.9237  0.9956  1.0007
   ZnK     4.71    1.02   0.0383   0.7944  1.0061  1.0171
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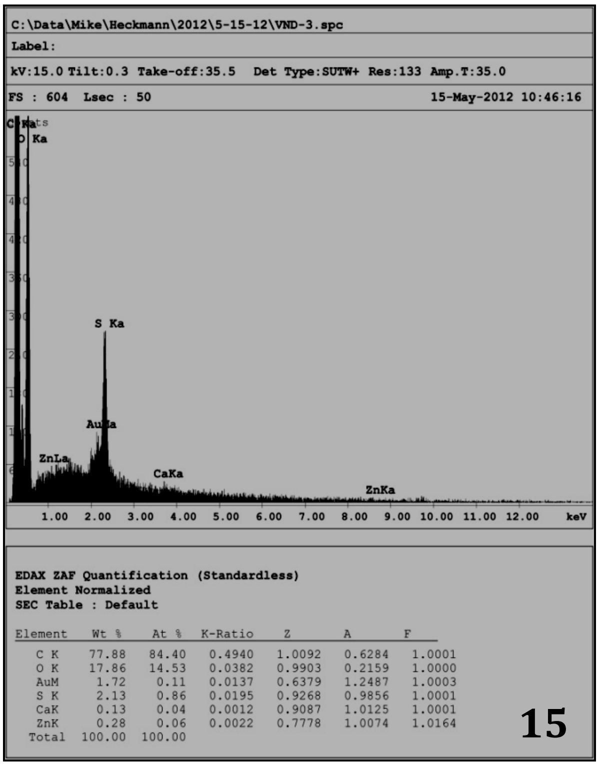
14



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Element Normalized
SEC Table : Default

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   O K    17.86   14.53   0.0382   0.9903  0.2159  1.0000
   AuM     1.72    0.11   0.0137   0.6379  1.2487  1.0003
   S K     2.13    0.86   0.0195   0.9268  0.9856  1.0001
   CaK     0.13    0.04   0.0012   0.9087  1.0125  1.0001
   ZnK     0.28    0.06   0.0022   0.7778  1.0074  1.0164
  Total  100.00  100.00
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15

Figure 13. EDXA printout for the posterior region of *D. paradoxum.* **Figure 14.** Same as 12 but anterior region. **Figure 15.** EDXA scan of an egg for *D. paradoxum.*

Discussion

There continues to be interest in the scientific community for research concerning this unique symbiotic parasite. *Diplozoon paradoxum* is common in freshwater fishes in Asia and Europe. It is commonly found in the gills (ectoparasite) of European cyprinid fishes. To date, there have been no published articles on the presence of *Diplozoon* in North and South America. It will be expected that it will be found in the America's due to international transport especially within the Tropical Fish Industry (Varadi, 2001). An example of these tropical imports would be snakehead fish, *Channa* sp. (Böhme, 2004; Courtenay and Williams, 2004; Britz, 2007; Zambito, 2011) and grass carp. Carp are common hosts for *D. paradoxum* and this cyprinid and close relatives are moved throughout the world (Yamaguti, 1963). Grass carp *(Ctenopharyngodon idella),* native to the orient and Asia have also been moved and introduced throughout the world including the United States. The senior author published several papers on a parasite introduced into the North America area due to grass carp (Heckmann, 1996; 1997; 2000). *C. idella* was introduced into the USA for aquatic weed control.

Other research interest for *D. paradoxum* includes chemical treatment for the parasite (Schmahl and Taraschewski, 1987) and new fish hosts such as the bleak *(Alburnus alburnus)* (Koyun and Altunel, 2007; Kagel and Taraschewski, 1993) and *Anguilla anguilla* (Altunel, 1979). Other authors have extended the range for the ectoparasite (Aydogdu and Altunel, 2002; Chubb, 1977; Gelnar et al., 1994; Halvorsen, 1969; Molnar and Jalali, 1992; Molnar and Szekely, 1995; Oguz, 1991).

Surveys and check lists for all parasites including the monogenea are numerous (Yamaguti, 1963; Bychovskaya-Pavlovskaya, 1962; Kennedy, 1974; Bauer, 1984; Hoffman, 1970).

This is the first known published study for electron optics, both SEM and EDXA, of *D. paradoxum.* The morphological features are prominent on the surface of the ectoparasite using SEM especially the attachment structures.

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