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DOI 10.2478/helm-2018-0037

HELMINTHOLOGIA, 56, 1: 11 – 21, 2019

Structure and morphometrics of *Ancyrocephalus paradoxus* (Monogenea: Ancyrocephalidae) from *Sander lucioperca* (Percidae) in Czechia

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Article info

Received August 17, 2018
Accepted September 20, 2018

Summary

New morphometric data, including details of the copulatory system and attachment structures, as well as inner organs are provided for *Ancyrocephalus paradoxus* Creplin, 1839. Scanning electron microscopy reveals new information of the body shape, position of the cephalic organs' openings, and structure of anchors, as well as differences in the in anchors' structure in adults and sub-adults of *A. paradoxus.* Energy dispersive analysis for X-ray was conducted for the first time for anchors in Monogenea and revealed structural differences between different parts of the anchors in two age groups.
Keywords: *Ancyrocephalus paradoxus*; morphometrics; SEM; EDXA

Introduction

Ancyrocephalus paradoxus Creplin, 1839, the type species of genus *Ancyrocephalus*, is an euryhaline monogenean, known from Baltic to Aral Seas, Black and Azov seas, the Caspian, and the Mediterranean. Gusev (1985) reported that *A. paradoxus* is distributed worldwide together with its host. It has been reported from Europe and Asia. In the Ukrainian territory of Europe, this species was recorded from many localities including Danube, Dniester, Tisza, Prypyat and Dnieper rivers and its reservoirs, and the Black and Azov Sea basins (Kulakivska, 1954, 1973, 1974; Komarova, 1964, 1972; Pashkevichute, 1971; Iskov & Koval, 1973; Koval, 1978; Solonchenko, 1982; Rubtsova, 2003; 2015). In Poland *A. paradoxus* was reported from Lakes Jamno, Lebsko, Dargin, Gulf of Gdansk, Vistula Lagoon and from the Pomeranian Bay of the Baltic Sea (Wierzbicki, 1970; Rolbiecki & Rokicki, 1996; Rolbiecki, 2003; Zaostrovtseva, 2009; Bielat *et al*, 2015). It was registered in Great Britain (Brewster, 2016); Hungary (Molnar *et al*., 2016); Czechia (Mendoza-Palmero *et al*., 2015; Acosta *et al*.; 2017); Romania (Cojocary, 2009); Azerbaijan (Ibragimov & Shakaralieva, 2014); Russia (Izyimova 1958; Gusev, 1985; Zharikova *et al*., 2002; Rumyantsev, 2004); Turkey (Ozturk & Ozer, 2014), Iran (Pazooki & Masoumian, 2012). Chubb (1977) studied the occurrence of *A. paradoxus* in different climate zones. Starovoitov (1989, 1999) studied different ecological aspects and relationships in the host-parasite system. Molnar *et al*. (2016) provided histological investigations of *A. paradoxus.*

In spite of these extensive reports, the description of *A. paradoxus* was very brief and a few redescriptions and records were lacking some basic morphometric data and accuracies (Ergens, 1966; Bykhovsky & Nagibina, 1970; Lom & Ergens, 1970 and Gusev, 1985). Metal analysis have never been performed for *A. paradoxus*. Though it was successfully used for differentiation of two age stages in Polystomatidae (Rubtsova & Heckmann, 2017), it was never carried out for different gallium cuts of the anchors in Monogenea, accomplished with SEM. In the present study, we provide metric parameters according to Gusev (1985) that now are widely used in studies of Monogenea (Šimková *et al*., 2013; Acosta *et al*.,

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2018). For instance, “complete anchor length” in Ergens (1966) is what was later accepted as ventroapical length (for four-anchored monogeneans) (Gusev, 1985). Thus, the addition and expansion of the morphometric data, the results of metal analysis of the anchors, as well as the results of gallium cuts of different parts of anchors of *A. paradoxus* in two age groups prompted this study and represents its major contributions.

Material and Methods

Sample collection

Fish was collected with gill-netting – a 22 mm mesh benthic gillnet (15 m length, 1.5 m height) was installed across the pond (5 m from the bank) for 3 h during the day and controlled every 0.5 h. Sampling time: 11:00−12:00 h. All fish were transported alive in aerated barrels to the laboratory of the Institute of Vertebrate Biology, Czech Academy of Sciences (Brno), where they were transferred to a 1 m^3 outdoor holding basin (separate basin for each sampling method). Before dissection, the standard length (SL) of each fish was determined and gills were examined for monogeneans. All fish were dissected within 48 h of sampling (Kvach *et al*., 2016). Thirty-two mature specimens of *Sander lucioperca* age 3+, SL 26.9 (21.4 – 30.5 cm) were studied for the presence of *Ancyrocephalus paradoxus* at Cezarka pond, Vodnany, Czechia (49°08’47.0”N 14°11’28.7”E) on 17 – 18 October 2017. Twenty specimens of *A. paradoxus* were used for morphometric studies, and 8 specimens were used for SEM and metal analysis.

Light microscopy

Worms were stained in Mayer’s acetic carmine, destained in 4 % hydrochloric acid in 70 % ethanol, dehydrated in ascending concentrations of ethanol (12 hr. each), cleared in 100 % xylene and then in 50 % Canada balsam and 50 % xylene (12 hr. each). Whole worms were then whole mounted in Canada balsam. Measurements of sclerotized parts in the present study were made using the scheme shown at Fig. 1. The range is followed by the mean values between parentheses.

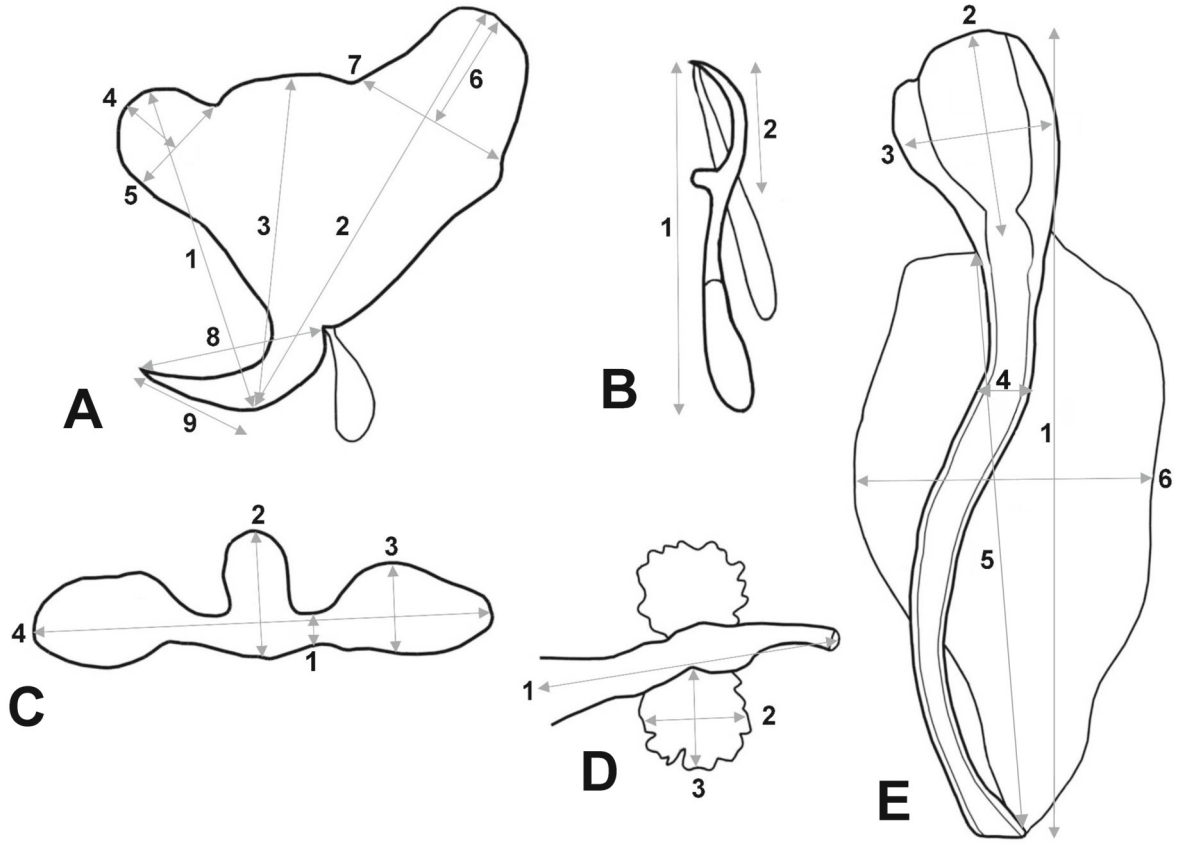


Fig. 1. Scheme of measurements of sclerotized parts of *Ancyrocephalus paradoxus.*
A – ventral/dorsal anchor (1 – dorsoapical length, 2 – ventroapical length, 3 – base part length, 4 – inner root length, 5 – inner root width, 6 – outer root length, 7 – outer root width, 8 – blade length, 9 – point length); B – marginal hooklet (1 – marginal hooklet length, 2 – marginal hooklet blade length); C – ventral/dorsal bar (1 – length in the narrowest part, 2 – length of the middle extension, 3 - length of the lateral extensions); D – vagina (1 – vagina length, 2 – comb-like structures length, 3 - comb-like structures width); E – copulatory organ (1 – copulatory organ total length, 2 – copulatory organ wide part length, 3 – copulatory organ wide part width, 4 – copulatory organ tube diameter, 5 – accessory piece of copulatory organ length, 6 – accessory piece of copulatory organ width)

Scanning Electron Microscopy (SEM)

Samples of parasites fixed and stored in 70 % ethanol were processed following standard methods [Lee, 1992] which included critical point drying (CPD) in sample baskets and mounted on SEM sample mounts (stubs) using conductive double-sided carbon tape. Samples were coated with gold and palladium for 3 minutes using a Polaron #3500 sputter coater (Quorum [Q150 TES] www.qurumtech.com) establishing an approximate thickness of 20 nm. Samples were placed and observed in an FEI Helios Dual Beam Nanolab 600 (FEI, Hillsboro, Oregon) Scanning Electron Microscope with digital images obtained in the Nanolab software system (GEI, Hillsboro, Oregon) and then transferred to a USB for future reference. Samples were received under low vacuum conditions using 10 KV, spot size 2, 0.7 Torr using a GSE detector.

Energy Dispersive X-ray Analysis (EDXA)

Standard methods were used for preparation similar to the SEM procedure. Specimens were examined and positioned with the above SEM instrument, which was equipped with a Phoenix energy-dispersive x-ray analyzer (FEI, Hillsboro, Oregon). X-ray spot analysis and live scan analysis were performed at 16Kv with a spot size of five and results were recorded on charts and stored with digital imaging software attached to a computer. The TEAM (Texture and Elemental Analytical Microscopy), a modification of the EDAX (Energy Dispersive analysis for X-ray) system, software system (FEI, Hillsboro, Oregon) was used. The data included weight percent and atom percent of the detected elements following correction factors.

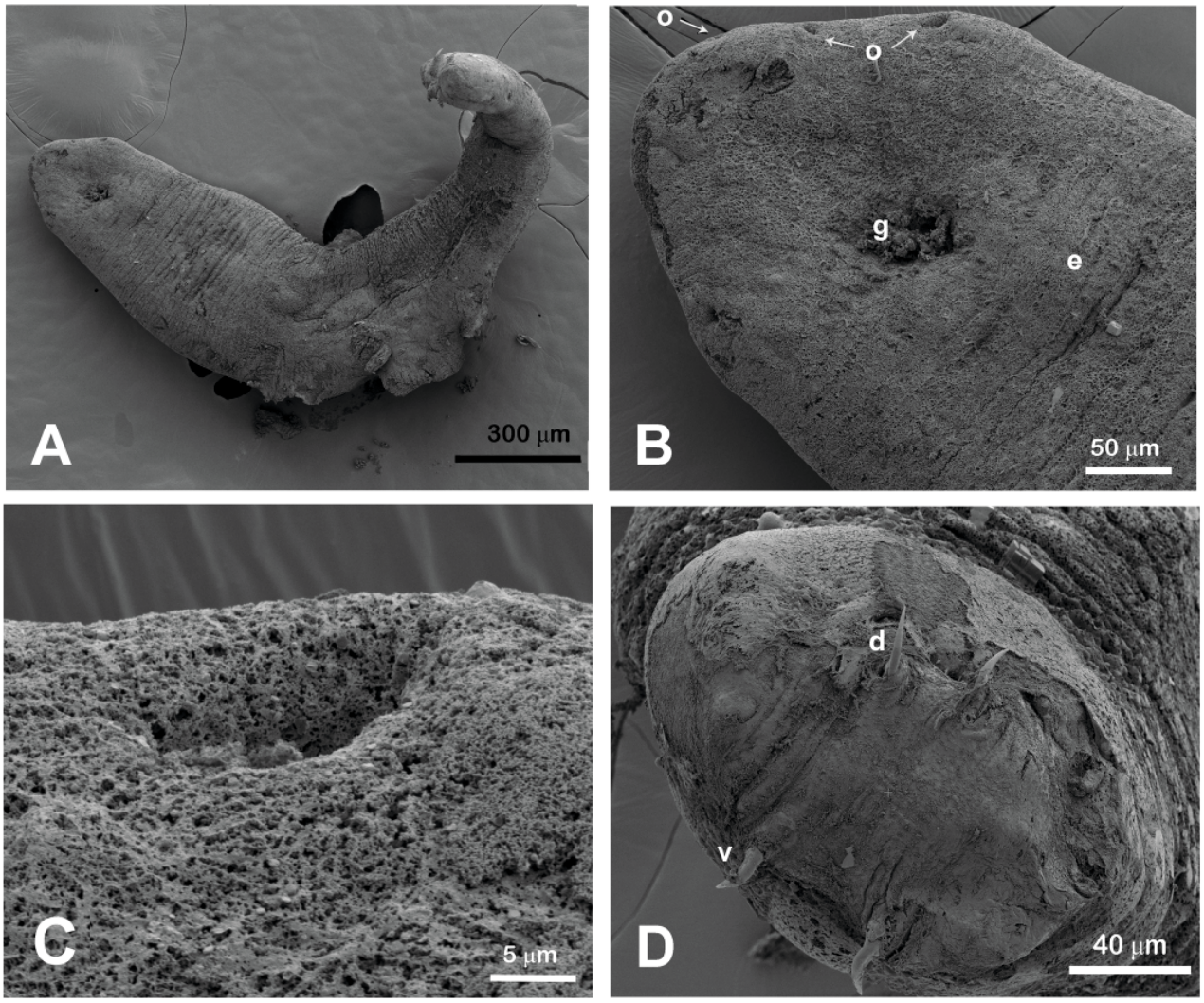


Fig. 2. SEM of an adult of *Ancyrocephalus paradoxus*.
A – whole body, ventral view; B – prohaptor of *A. paradoxus* (o – cephalic gland openings; g – mouth groove, e – an elevation in the area of protrusion of the copulatory organ); C – one of the six cephalic gland openings of the prohaptor; D – opisthohaptor of *A. paradoxus*, an end view (v – ventral anchor; d – dorsal anchor)

Ethical Approval and/or Informed Consent

The research related to animals has been complied with all relevant national regulations and institutional policies for the care and use of animals.

Results

Infection levels

All hosts were highly infected with *A. paradoxus* with intensity of 138 (101 – 188) parasites per fish.

Measurements of A. paradoxus

In the present study, we provide detailed measurements of hard parts and soft inner organs of mature specimens of *A. paradoxus* (Table 1), namely providing the metrical information on haptor, peduncle, detailed measurements of dorsal and ventral anchors, that include ventro- and dorsoapical lengths, roots parameters, base, blade and point lengths. We also provide marginal hooklet metric parameters, as well as copulatory system parameters, that include details of copulatory organ tube, accessory piece, vaginal tube and its accessory parts, as well as pharynx, ovary and testis parameters.

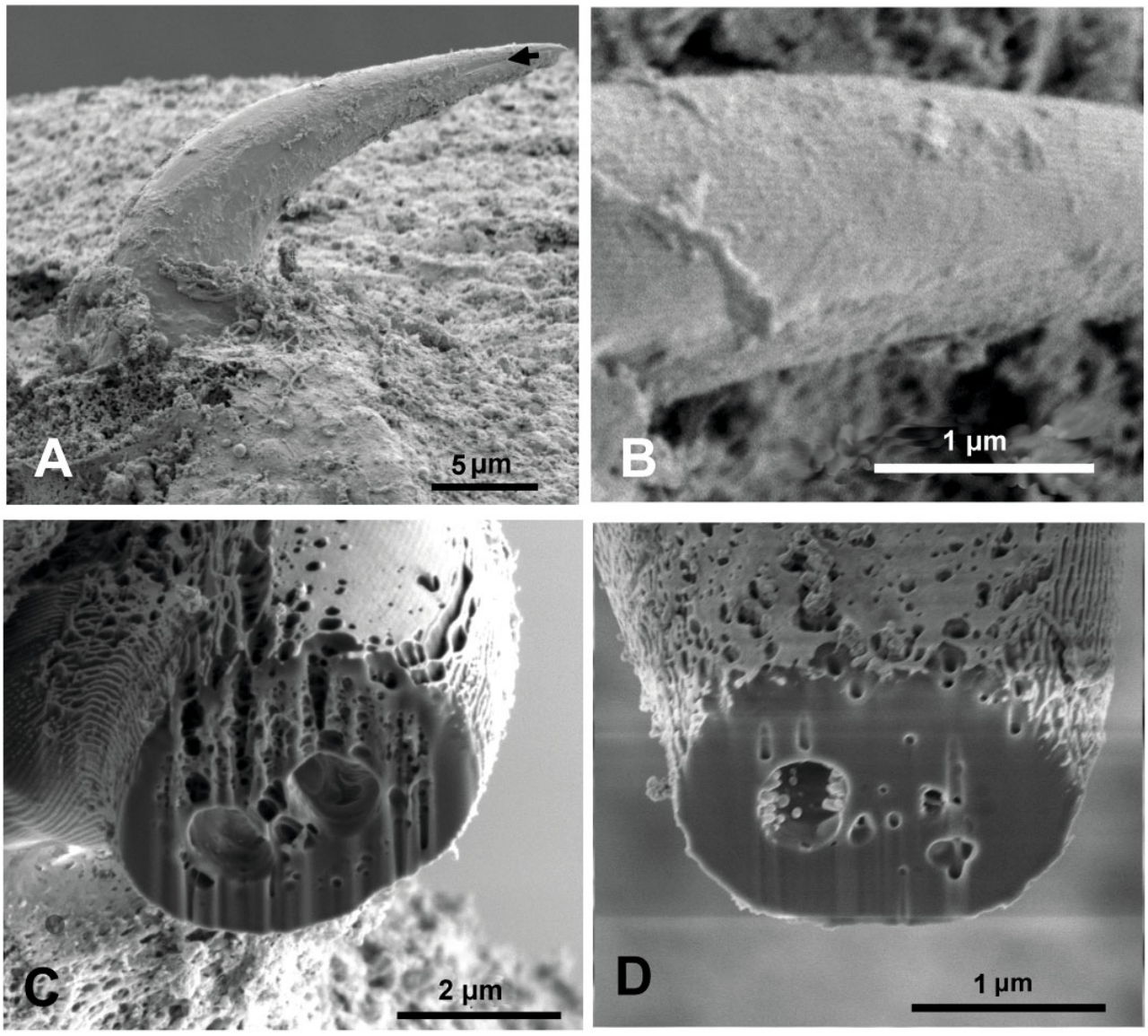


Fig. 3 SEM of an anchor of an adult of *Ancyrocephalus paradoxus*.
A – note longitudinal depression (arrow) in the distal part of the blade; B – an approximate view of the anchor surface. Note the longitudinal stiffeners; C – a gallium cut of the anchor, close to the base. Note the inner porous structure; D – a gallium cut of the anchor, close to its tip. Note dense, homogeneous, calcified tissue of the anchor on the cut

Table 1. Comparative metric characteristics of *Ancyrocephalus paradoxus* from *Sander lucioperca*, in mm.

	Ergens, 1966	Bykhovsky & Nagibina, 1970	Gusev, 1985	Present study
Body length	2.34 – 4.68	1.50 – 3.50	4.7	1.965 (1.750 – 2.125)
Body width	0.39 – 0.78	0.50 – 0.70	0.8	0.505 (0.375 – 0.750)
Haptor length	0.15 – 0.39	–	–	0.034 (0.028 – 0.045)
Haptor width	0.15 – 0.49	–	–	0.048 (0.040 – 0.063)
Peduncle length	– ***	–	–	0.096 (0.075 – 0.138)
Peduncle width	–	–	–	0.048 (0.030 – 0.063)
Ventral anchor:				
dorsoapical length *	–	–	–	0.042 (0.038 – 0.051)
ventroapical length	0.056 – 0.063	0.054 – 0.057	0.050 – 0.063	0.057 (0.053 – 0.063)
base length	–	–	–	0.039 (0.035 – 0.043)
inner root length	d.**	–	–	0.008 (0.006 – 0.011)
inner root width	–	–	–	0.014 (0.013 – 0.015)
outer root length	d.	–	–	0.008 (0.001 – 0.011)
outer root width	–	–	–	0.017 (0.015 – 0.018)
blade length	0.023 – 0.026	–	–	0.022 (0.018 – 0.025)
point length	–	–	–	0.009 (0.007 – 0.013)
Ventral bar length 1	0.003 – 0.005	–	–	0.005 (0.003 – 0.008)
Ventral bar length 2	0.007 – 0.016	–	0.009 – 0.014	0.010 (0.006 – 0.013)
Ventral bar length 3	0.011 – 0.014	–	–	0.012 (0.010 – 0.013)
Ventral bar width	0.037 – 0.060	0.045 – 0.050	0.045 – 0.060	0.047 (0.038 – 0.060)
Dorsal anchor:				
dorsoapical length	–	–	–	0.041 (0.038 – 0.043)
ventroapical length	0.050 – 0.060	0.052 – 0.060	–	0.057 (0.055 – 0.060)
base length	–	–	–	0.033 (0.028 – 0.038)
inner root length	d.	–	–	0.010 (0.008 – 0.013)
inner root width	–	–	–	0.015 (0.014 – 0.015)
outer root length	d.	–	–	0.008 (0.002 – 0.015)
outer root width	–	–	–	0.020 (0.015 – 0.023)
blade length	0.023 – 0.028	–	–	0.027 (0.025 – 0.028)
point length		–	–	0.013 (0.010 – 0.015)
Dorsal bar length 1	0.002 – 0.004	–	–	0.005 (0.004 – 0.008)
Dorsal bar length 2	0.007 – 0.008	–	0.008 – 0.012	0.009 (0.007 – 0.015)
Dorsal bar length 3	0.007 – 0.011	–	–	0.011 (0.009 – 0.013)
Dorsal bar width	0.049 – 0.060	0.060 – 0.064	0.060 – 0.070	0.050 (0.043 – 0.055)
Marginal hooklet length		0.018 – 0.020	0.017 – 0.020	0.021 (0.015 – 0.023)
Marginal hooklet blade length	–	–	–	0.007 (0.005 – 0.009)
Copulatory organ total length	–	–	–	0.148 (0.133 – 0.163)
Copulatory organ wide part length	–	–	–	0.036 (0.028 – 0.043)
Copulatory organ wide part width	–	–	–	0.026 (0.023 – 0.030)
Copulatory organ tube diameter	–	0.008 – 0.010	0.006 – 0.010	0.007 (0.005 – 0.009)
Copulatory organ tube length	–	0.10	0.13 – 0.16	–
Accessory piece length	–	–	–	0.088 (0.063 – 0.100)
Accessory piece width	–	–	0.070	0.060 (0.055 – 0.065)
Vaginal tube length	–	–	0.040 – 0.050	0.050 (0.038 – 0.058)
Vaginal tube diameter	–	–	0.010	0.010 (0.008 – 0.011)
Comb-like growths if vaginal tube length	–	–	–	0.015 (0.015 – 0.015)
Comb-like growths if vaginal tube width	–	–	0.020	0.019 (0.018 – 0.020)
Pharynx length	–	0.14 – 0.16	–	0.138 (0.130 – 0.146)
Pharynx width	–	–	–	0.109 (0.104 – 0.114)
Ovary length	–	0.16 – 0.20	–	0.139 (0.125 – 0.156)
Ovary width	–	–	–	0.166 (0.125 – 0.208)
Testis length	–	0.16 – 0.18	–	0.140 (0.125 – 0.156)
Testis width	–	–	–	0.177 (0.166 – 0.187)

* – see Fig. 1 for the scheme of measurements and abbreviations

** d. – data provided in literature are doubtful (see Discussion part)

*** data not available

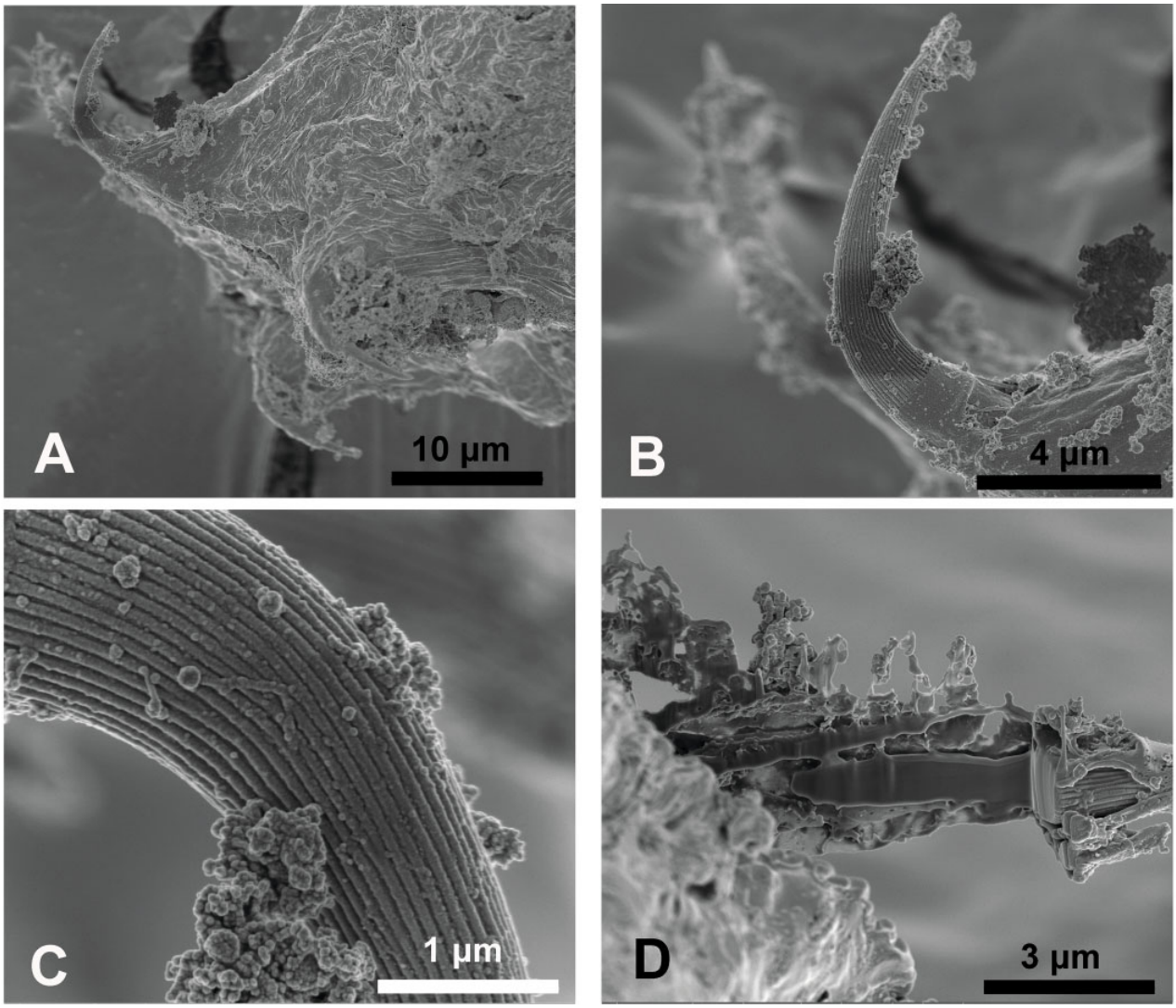


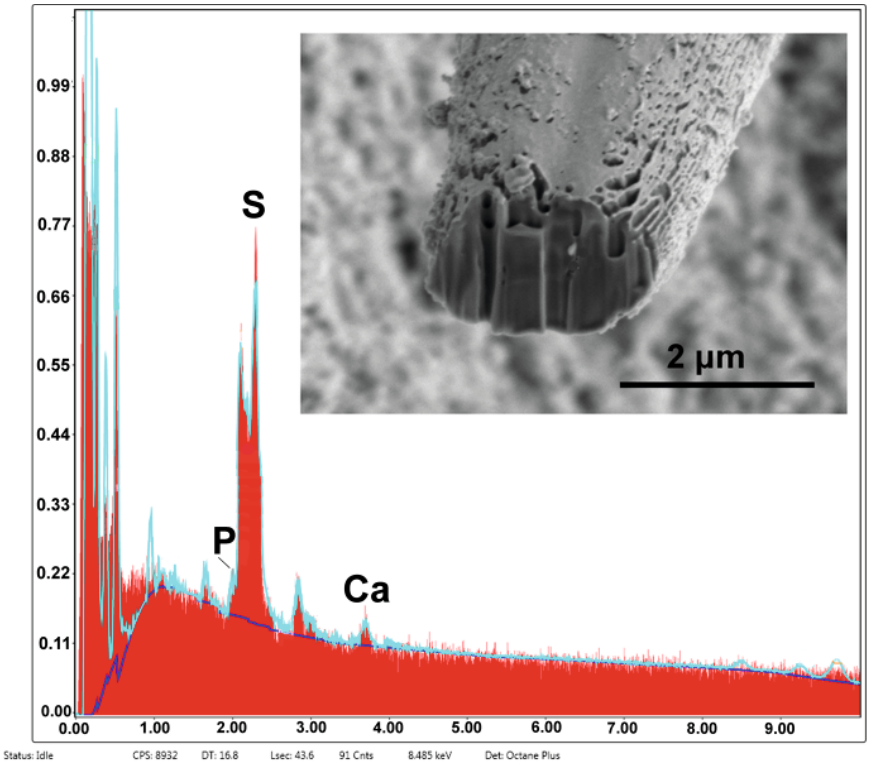
Fig. 4 SEM of a sub-adult of *Ancyrocephalus paradoxus*.
A – opisthohaptor of *A. paradoxus*; B – an anchor of *A. paradoxus.* Note the longitudinal stiffeners; C – an anchor of *A. paradoxus* (enlarged). Note the longitudinal stiffeners; D – frontal plane gallium cut of an anchor base of *A. paradoxus*

Results of scanning electron microscopy (SEM)

SEM reveals the following features in the anatomy of *A. paradoxus*. The forebody has a characteristic broad rhomboid-shaped prohaptor (Fig. 2A). A close SEM microphotograph of a prohaptor (Fig. 2) shows the outer structure of three pairs of cephalic gland openings, mouth groove and an elevation in the area of protrusion of the copulatory organ (Figs. 2B, 2C). The haptor of sub-adult (Fig. 4A) is compared to its juvenile shape with outstanding anchors and almost square end of a haptor, opposite to the haptor of an adult, with rectangular shape and relative sizes of anchors being twice as small as the haptor itself (Fig. 2D).

The cross-section of the point of the anchor also differs in two different age stages. In the adult worm, it has an oval shape; the sub-adult has a triangular shape with rounded edges or near circular (Figs. 4, 5). In spite of the opinion of Ergens (1966) that the ventral and dorsal anchors are similar, they are clearly different in their thickness; see the distal end view of the haptor (Fig. 2D) and compare ventral and dorsal ones. The anchors themselves differ by their surface structure. In adults and sub-adults, they possess longitudinal ribs (compare Fig. 3B, Fig. 4 B and C). In adults, the ribs are more numerous and not so pronounced.

The blade of anchors has a characteristic longitudinal depression (Fig. 3, A). A close SEM microphotograph of the surface of the anchor (Fig. 3B) demonstrates that its entire surface is covered with uniform longitudinal ribs. Figs. 3 C and 3 D show gallium cuts in two different parts of the anchor – a thick part that is closer to the base (Fig. 3 C) and a narrow part at the distal point of the anchor (Fig. 3D). The central part of the blade close to the base



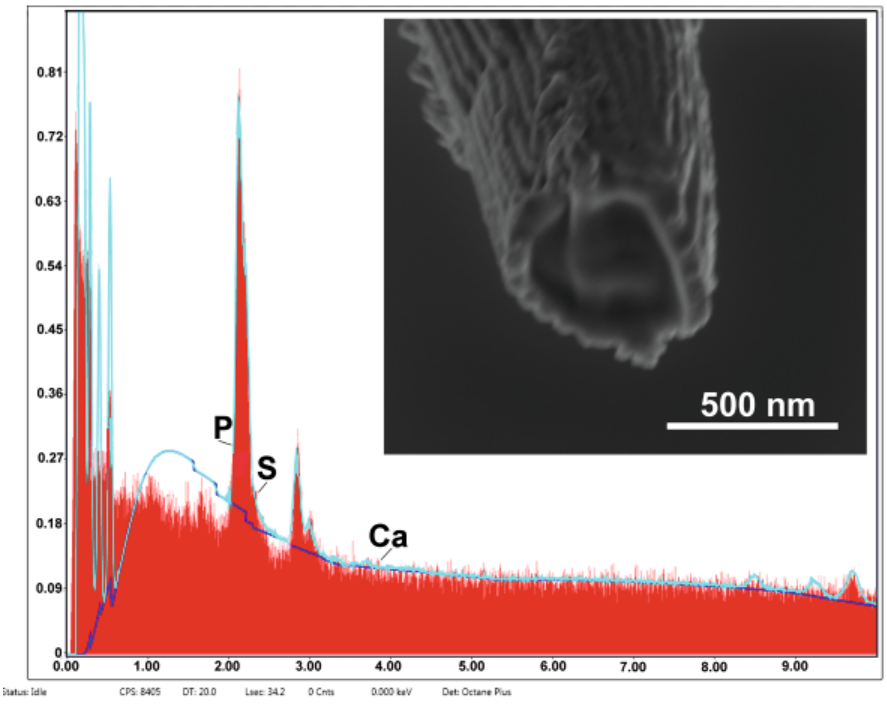
Element	Weight %	Atomic %	Error %	Net Int.	K Ratio
C K	24.92	38.67	8.89	228.25	0.121
N K	20.5	27.28	15.22	108.93	0.0637
O K	20.43	23.79	10	229.93	0.0661
Ga L	1.18	0.31	17.25	15.69	0.0084
Mg K	0.22	0.17	62.14	7.4	0.0015
P K	1.11	0.67	18.58	33.08	0.009
Au M	15.37	1.45	9.09	189.73	0.1134
S K	10.72	6.23	5.03	299.54	0.0855
Pd L	3.95	0.69	29.15	44.59	0.026
Ca K	1.6	0.74	32.15	25.05	0.0127

Fig. 5. An X-ray elemental analysis of the distal part of anchor of an adult of *A. paradoxus*.

has cavities and pores. Two bigger central cavities, apparently extend along the length of the anchor. A multitude of smaller pores of different sizes are located randomly, but mostly at the external curvature of the anchor's shaft. On the other hand, the distal part of the anchor (Fig. 3D) has a dense structure devoid of pores demonstrating the strength of the hook in this section. Scanning electron micrographs of the studied areas are shown on Figs 4 and 5 as well. The general shape of the sub-adult anchor's cut demonstrates its softness (Fig. 6), while in the mature specimen the structure is solid (Fig. 5). Visually and by chemical analysis, drastic chemical and morphological changes in the attachment structures are well demonstrated. The characteristic longitudinal depression of the blade of anchors (Fig. 3, A) resembles a trough on the blade of hunting knives, and, apparently, providing an additional elasticity and hardness to the anchor Anchors of both ages also have longitudinal ribs that apparently, give the anchor additional strength.

Results of the EDXA study

Other novel studies used in the present work is Energy Dispersive X-ray Analysis of different parts of the anchors, never performed before for anchors in Monogenea. An X-ray elemental analysis of the middle part and distal part of anchors of *A. paradoxus* in two different life stages are compared, see Table 2 for % weight of Mg, P, S and Ca. Common elements (C, H, O) that are present in all protoplasm and processing elements (Ga, Pd, Au) are omitted.



Element	Weight %	Atomic %	Error %	Net Int.	K Ratio
C K	23.29	36.13	9.12	224.2	0.1197
N K	21.26	28.28	14.69	117.38	0.0691
O K	22.53	26.23	9.8	254.92	0.0737
Ga L	1.17	0.31	15.59	15.4	0.0083
Mg K	0.21	0.16	65.16	6.93	0.0014
P K	0.91	0.55	22.34	26.89	0.0073
Au M	15.22	1.44	8.83	185.87	0.1119
S K	9.1	5.29	5.61	252.4	0.0726
Cl K	0.09	0.05	99.99	2.16	0.0007
Pd L	4.6	0.81	26.74	51.77	0.0304
Ca K	1.61	0.75	31.5	25.1	0.0129

Fig. 6. An X-ray elemental analysis of the distal part of anchor of a sub-adult of *A. paradoxus*.

The uncut anchor demonstrated dominating high level of sulfur. We provide a comparison of chemical elements of gallium cut anchors of a mature adult specimen of *A. paradoxus* and immature sub-adults for the first time in Monogenea. There is a clear tendency for increased phosphorus weight percentage in an immature (sub-adult) specimen compared to mature specimens where the sulfur is about 10 times higher. Calcium is the prevalent element in the distal part of anchor's blade of sub-adult and dominates in the distal part of anchor in sub-adult in comparison to the middle part, but in adult calcium is prevalent in the middle part in comparison with the distal part of the anchor (Table 2). Figures 4 and 5 show results of the spectrum analysis of the gallium cuts of the distal parts (tips) of anchors.

Discussion

Measurements of A. paradoxus

By providing detailed measurements of hard parts and soft inner organs of mature specimens of *A. paradoxus* (Table 1), we are filling a large gap in a very scant information in the redescriptions by Ergens (1966), Gusev, Kulemina (1971) and Gusev (1985).

Influence of the method of preservation and fixation on measurements

Parameters of soft body structures, especially body length and width in Monogenea can change depending on methods of preservation and fixation. As a rule, hard parts of monogeneans remain

Table 2. X-ray scans of Ga cuts of anchors of *Ancyrocephalus paradoxus*.

	Sub-adult		Adult		Adult
Element	Distal part of anchor's blade	Middle part of anchor's blade	Distal part of anchor's blade	Middle part of anchor's blade	Uncut anchor
Magnesium (Mg)	0*	0.25	0.22	0.2	0.09
Phosphorus (P)	0.21	1.81	1.11	1.62	0.21
Sulfur (S)	0.16	0.31	10.72	14.6	5.49
Calcium (Ca)	0.43	0.29	1.6	2.74	0.04

* – see spectrum figures (Figs. 4 and 5); numbers represent % wt

the same in different types of preservation and fixation. The worm keeps its "3D" body shape when preserved in alcohol for SEM or in acetic carmine for staining before mounting in Canada balsam (see SEM, Fig. 2A). When fixed in ammonia picrate or glycerin jelly (that allows better vision of sclerotized structures), the body and inner soft organs appear flattened which influences measurements. Describing the shape of haptor in the present study in non-pressed state, its width appears almost equal to the length, while in glycerin jelly whole mounts, it has clear rectangular shape with the width appearing noticeably larger than length, as it was drawn in Bykhovsky & Nagibina (1970). For taxonomic purposes, we consider glycerin jelly fixation as an optimal procedure for small monogeneans, without strong pressure, just under natural weight of the coverslip.

Ergens (1966) redescribes *A. paradoxus* from fishes in Central Europe (Danube, Elbe and Oder rivers) and provides drawings and limited measurements (see Table 1), that lacked details which we clarify in the present study. In his redescription, Ergens (1966) did not describe the inner soft anatomy of *A. paradoxus*, and focused only on the measurements and morphology of sclerotized parts of copulatory system and haptor. Ventral and dorsal pairs of anchors, named "first pair" and "second pair", did not provide an understanding of the position of each pair of anchors in the haptor in his study (Ergens, 1966). Measurements of anchors provided in his paper were not complete. Moreover, he used untraditional metric parameters. For instance, he provided measurement of the length of inner and outer roots that did not, however, correspond to the length of root itself, but the root and a base of the anchor (see Fig. 1 A in Ergens, 1966). In the present study we are providing this information, according to the scheme of measurements (see Fig 7 (5) in Gusev, 1985). This parameter is critical for species definitions in Monogenea, because some parts of the anchor keep growing during the lifespan of the worm (Gusev & Kulemina, 1971). The length of the blade named "point" in Ergens (1966) is practically a different anchor parameter, according to Gusev (1985) (see Fig. 1). In his redescription Ergens (1966) did not distinguish dorsal and ventral bars, calling them first pair and second pair. From his drawings, we assume that he considered the ventral set of anchors as the first pair and its bar (the ventral bar) and the second pair as the dorsal anchors pair and its bar (the dorsal bar).

Bykhovsky & Nagibina (1970) provided a redescription of *A. paradoxus* with some information on inner anatomy of the worm that included diameters of pharynx, ovary and testis (Table 1) together with few measurements of sclerotized parts (copulatory organ tube length and diameter). For both ventral and dorsal anchors, they provide a single measurement, a "ventroapical length". Information on the sizes of ventral and dorsal bars was given for the bar's width only (mistakenly called length).

Gusev (1985) in his "Keys to freshwater fish parasites of USSR" (1985) gave brief information on the main metric parameters of *A. paradoxus*, that included body width and length, a single metric parameter (dorsoapical length) for both ventral and dorsal anchors, considering them to have similar morphology, measurements of bars, hooklets and copulatory system. In spite of giving only one parameter for anchors, Gusev (1985) gave a comprehensive set of measurements of *A. paradoxus* – type anchors [see Fig. 7.5 in Gusev (1985)], that included proper measurements for inner and outer roots, blade dorsoapical and ventroapical lengths, that we are currently using as a base in the present study (Fig. 1).

Cephalic organs

We provide SEM photographs of cephalic organs' openings in *A. paradoxus*. Bakke *et al*. (2004) reported a high number of sensilla distributed ventrally around the oral pore and the region of the penis, that probably indicates that the sensilla serve to orient the gyrodactylid during feeding and copulation. Bakke *et al*. (2004) claimed these sensilla might have a different function to those sensilla distributed around the cephalic lobes, which must play a crucial role when transferring between hosts and moving over the host's epidermis (Bakke *et al*, 2004).

EDXA of different parts of anchors from two different age groups of A. paradoxus

The comparison between gallium cuts from medial and distal parts of the anchor show that at the middle part, this structure is more flexible, while it is the hardest and calcified terminally. The amount of calcium (Ca), phosphorus (P), and sulfur (S) (Fig. 4) is emphasized because they metabolize into hardened structures as found in mammalian teeth. Same tendencies were recently reported in the attachment structures of acanthocephalans (Heckmann *et al*.,

2012). The calcium and phosphorus form a rigid phosphate apatite similar to the enamel of mammalian teeth with disulfide bonds (cysteine) enhancing the strength of the structure. The enamel of mammalian teeth is over 95 % inorganic matter representing the hardest tissue in the body (Heckmann *et al*., 2012). The levels of structural minerals especially calcium and phosphorus at the central part of the anchor are too low to have any structural/attachment utility. These unique characters may be novel because they were simply not seen or reported by earlier researchers.

Conflict of Interest

Authors state no conflict of interest.

Acknowledgements

We are grateful to Dr. Markéta Ondračková, Institute of Vertebrate Biology ASCR, Brno (Czechia) for providing specimens of *A. paradoxum* for the present study. We are grateful to Michael Standing who provided his help with the SEM and EDAX processing in the BYU Electronic lab, Provo, Utah. This work was supported by an institutional grant from the Institute of Parasitic Diseases, Scottsdale, Arizona and financial support from Brigham Young University, Provo, Utah.

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