İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2020; 35(3): 64-68 • DOI: https://doi.org/10.26650/ASE2020668193

Research Article

Light and Scanning Electron Microscopic Observations on *Grillotia erinaceus* (Cestoda: Trypanorhyncha)

Ahmet Özer[1], Türkay Öztürk[1], Sevilay Okkay[1,2], Violetta Yurakhno[3], Julia Kornyychuk[3]

Cite this article as: Özer, A., Öztürk, T., Okkay, S., Yurakhno, V., Kornyychuk, J. (2020). Light and scanning electron microscopic observations on *Grillotia erinaceus* (Cestoda: Trypanorhyncha). *Aquatic Sciences and Engineering, 35*(3), 64-68.

ABSTRACT

In the present study, the plerocercoids of *Grillotia erinaceus* were obtained from the wall of the anterior oesophagus, stomach, pyloric caeca and liver of teleost Black Sea whiting *Merlangius merlangus* and adults were collected from the intestine of elasmobranch thornback rays *Raja clavata* caught by commercial fishing vessels off Sinop, Turkey. Standard parasitological investigation methods were applied and morphological diagnostic features of the whole parasite, bothria, scolex, tentacular armatures and tentacles were studied in detail using a light and Scanning Electron Microscope (SEM). The plerocercoids of this parasite had a total length of 5.96 mm on average and this was 21.6 mm on average in adults. The measurement data of all morphological diagnostics are provided and photomicrographs of each part of the parasite are presented. This study also provides the detailed morphological features of both plerocercoids and adults of *G. erinaceus* in *M. merlangus* and *R. clavata* for the first time in the Turkish coasts of the Black Sea.

Keywords: *Grillotia erinaceus*, Merlangius merlangus, raja clavata, black sea

ORCID IDs of the authors:
A.Ö. 0000-0002-2890-6766;
T.Ö. 0000-0001-5568-3214;
S.O. 0000-0003-4440-3525;
V.Y. 0000-0002-0571-6716;
J.K. 0000-0003-3095-6436

[1]Sinop University, Faculty of Fisheries and Aquatic Sciences, Sinop, Turkey

[2]Kocaeli University, Faculty of Agriculture and Natural Sciences, Kocaeli, Turkey

[3]A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Nakhimov Av., Sevastopol, Crimea

Submitted:
08.01.2020

Revision Requested:
02.03.2020

Revision Requested:
03.03.2020

Accepted:
08.03.2020

Online published:
20.03.2020

Correspondence:
Ahmet Özer
E-mail:
aozer@sinop.edu.tr

INTRODUCTION

Metazoan trypanorhynch cestodes, including the members of the genus *Grillotia* with approximately 277 valid species, are common parasites of marine fish and while sharks and rays host the adults, a wide variety of marine invertebrates and teleosts are infected by larval forms with low host specificity and a wide zoogeographical, or even cosmopolitan, distribution (Palm, 2004; Palm & Klimpel, 2007; Palm & Caira, 2008; Palm, Waeschenbach, Olson, & Littlewood, 2009). The cestode order Trypanorhyncha Diesing, 1863 is characterized by a scolex bearing 2 or 4 bothria and a tentacular apparatus, consisting of four retractile tentacles adorned with hooks as extensions of tentacle sheaths that are attached to four bulbs (Dollfus, 1942; Jones, Beveridge, Campbell, & Palm, 2004). These cestode parasites have a unique and complex attachment apparatus enabling their attachment and movement in the host organs through antagonistic bulbs and retractor muscles with the ability of invagination and retraction of them (Palm, Waeschenbach, Olson, & Littlewood, 2009). Larval and adult trypanorhynch cestodes have the same scolex morphology which makes accurate taxonomic diagnosis (Palm, 2004).

Grillotia plerocercoids are easily visible due to their white spherical or ovoid cysts and occur attached to the serosal surface or embedded in the wall of the oesophagus, stomach, pyloric caeca or intestine of their host fishes (Lubieniecki, 1976). Gadoid fishes are involved as the second intermediate hosts (Lubieniecki, 1976) and *Merlangius merlangus* has been reported to be the host of *Grilloia erinaceus* plerocecoids (Özer, Öztürk, Kornyushin, Kornyychuk, & Yurakhno, 2012; Özer, Öztürk, Kornyushin, Kornyychuk, & Yurakhno, 2014; Tepe, Oğuz, &

Heckmann, 2014). On the other hand, the adult trypanorhynchs including *G. erinaceus* are found in the spiral intestine of sharks and rays (elasmobranchs) (Palm, Yulianto, & Piatkowski, 2017). This species was reported as adults from 24 elasmobranch species and as pleurocercoids from 62 teleost fish species (see Menoret & Ivanov, 2012). Deardorff, Raybourne, & Mattis (1984) reported a decrease in the commercial value of affected stock caused by metacestodes (postlarvae and plerocerci) in the musculature of fishes.

The aim of the present study is to provide light and ultrastructural observations of a trypanorhynch plerocercoid, *Grillotia erinaceus* in whiting, *Merlangius merlangus* and adults from thornback rays *Raja clavata* collected from the Sinop coasts of the Black Sea, thus providing the first detailed observations on this species in Turkey.

MATERIAL AND METHOD

Specimens of *Grillotia erinaceus* were obtained from the wall of the anterior oesophagus, stomach and pyloric caeca of teleost Black Sea whiting *Merlangius merlangus* and from the intestine of elasmobranch thornback rays *Raja clavata* caught by commercial fishing vessels in the Black Sea off Sinop (N 42° 05' 68" E 35° 10' 55") in the period between May 2011 and April 2014. These fish were then examined for cestode parasites using standard methods. Cestode worms obtained from the mesenteries and stomach wall of whiting were either studied fresh or fixed in 10% formalin for morphological observations; subsequently, the formalin was replaced by 70% ethanol, then the tentacles of several worms were detached from scolexes and mounted in glycerine jelly (Chubb, Pool, & Veltkamp, 1987). Photographs of the mounted specimens and detached tentacles were taken using an Olympus BH2 microscope attached with a DP25 digital camera operated with digital imaging software. The measurements are in millimetres (mm), as is the range followed in parentheses by the mean. For SEM imaging, the worms were dehydrated in a graded ethanol series, placed in hexamethyldisilazane and allowed to dry (Shively & Miller, 2009). They were mounted on stubs and coated with gold and then SEM micrographs were taken using a Jeol JSM-6510LV scanning electron microscope at an accelerating voltage of 10kV. The terminology for the morphological characteristics and their measurements of trypanorhynchs follows Beveridge & Campbell (2007).

RESULTS AND DISCUSSION

Grillotia erinaceus plerocercoids were found in the form of white ovoid blastocysts approximately 6 mm long and were easily visible and occur attached to the serosal surface or embedded in the wall of the anterior oesophagus, stomach, pyloric caeca and liver of host fish *M. merlangus* (Figure 1). Free and encapsulated plerocercoids of *G. erinaceus* with a general view of scolex and bothria with 4 tentacles, external, internal and antibothridial surfaces of tentacular armatures as well as profiles of hooks on each tentacle are provided in Figure 2A-U.

Specimens of pregravid to mature *G. erinaceus* were found in the spiral intestine of the thornback rays *Raja clavata*. Adult worms were 21.6 mm long on average and the number of acraspedote proglottids was up to 32 per worm (Figure 3A). The Scolex were elongated and 6.2. mm long and 1.0 mm wide on average (Figure 3B). Tentacles elongated without basal swelling, measuring about 80 µm in diameter without hooks at base, about 60 µm without hooks in the metabasal region, the hook arrangements on tentacular apparatus are given in Figure 3C-E. All measurement data are provided in Table 1.

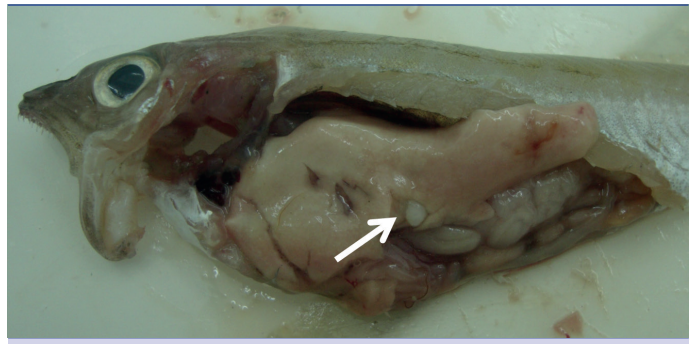

Figure 1. Whitish ovoid approximately 6mm diameter blastocyst (arrowed) attached to the wall of the *Merlangius merlangus* liver.

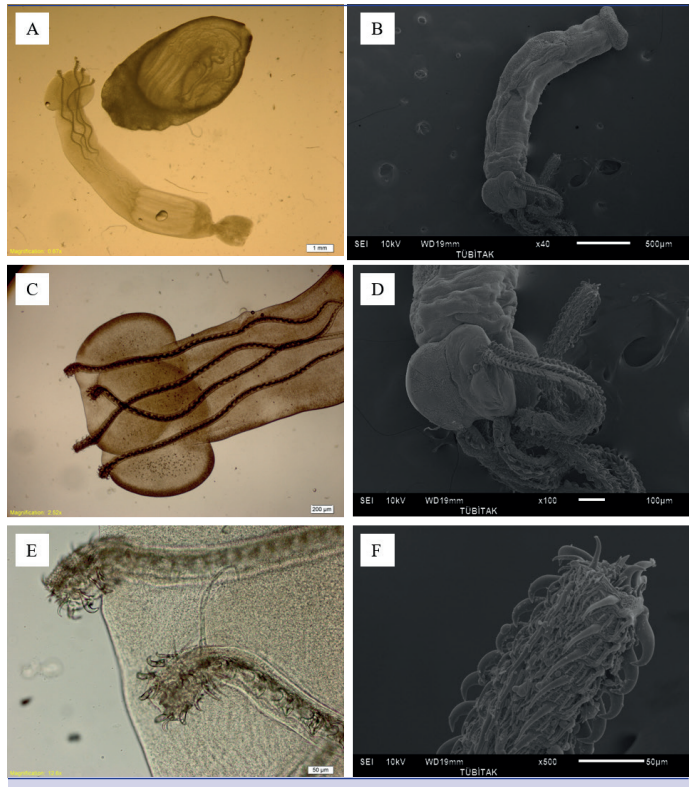

Figure 2. **A)** Free and encapsulated plerocercoids in *M. merlangus*, **B)** A general view of the scolex in SEM, **C)** Bothria with 4 tentacles, **D)** Bothria with fully exerted tentacles, **E)** A closer look at the tentacles, some parts of hooks outside the tube at tip and rest still inside, **F)** SEM observation of some exerted hooks at the tip of a tentacle.

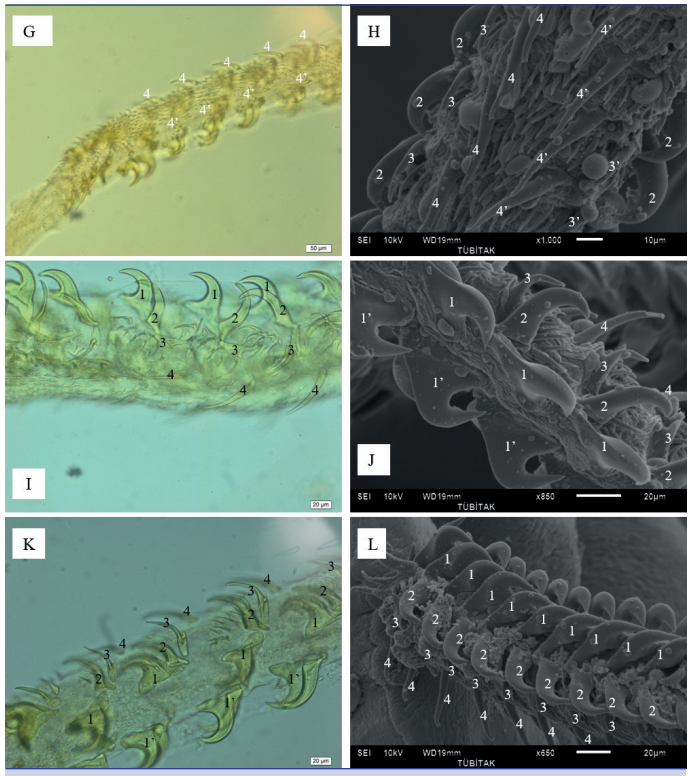

Figure 2. **G)** External surface, **H)** SEM observation of external surface, **I)** Antibothridial surface, **J)** SEM observation of the antibothridial surface and internal surface on the left hand side, **K)** Internal surface, **L)** SEM observation of internal surface.

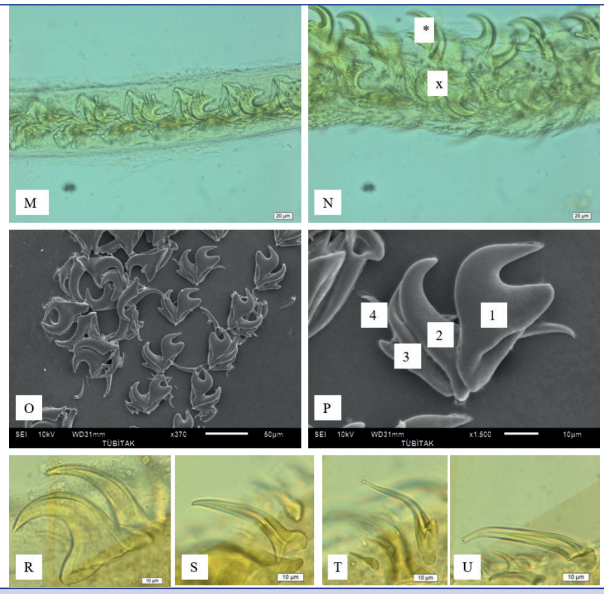

Figure 2. **M)** Hooks inside tentacle tube. **N)** Some parts of hooks outside (*) and some inside (x) the tentacle tube. **O)** SEM observation of hooks arrangements. **P)** Closer look at hook profiles 1-4 (SEM). **R)** Profile of hook 1. **S)** Profile of hook 2. **T)** Profile of hook 3. **U)** Profile of hook 4.

Grillotia erinaceus is one of the well-known species of the genus and the description here was made according to the morphometric data and figures presented by Lubieniecki (1976), Kornyushin & Solonchenko (1978), Beveridge & Campbell (2007). *Grillotia erinaceus* plerocercoids were found in the form of white spherical or ovoid blastocysts approximately 6 mm long and occurred attached to the serosal surface or embedded in the wall of the oesophagus, stomach, and pyloric caeca as was reported by Lubieniecki (1976) and Brickle, MacKenzie, & Pike (2006). This species was described initially from species of *Raja* Linnaeus, 1758 from the coast of Belgium but was subsequently reported from various species of rays on both sides of the north Atlantic (Dollfus, 1942) and it was first reported with only morphometric data by Kornyushin & Solonchenko (1978) in a cartilaginous fish *Raja clavata* in the Black Sea. Later, Özer, Öztürk, Kornyushin, Kornyychuk, & Yurakhno (2014) provided detailed information about its seasonal and host related occurrence at two southern and northern locali-

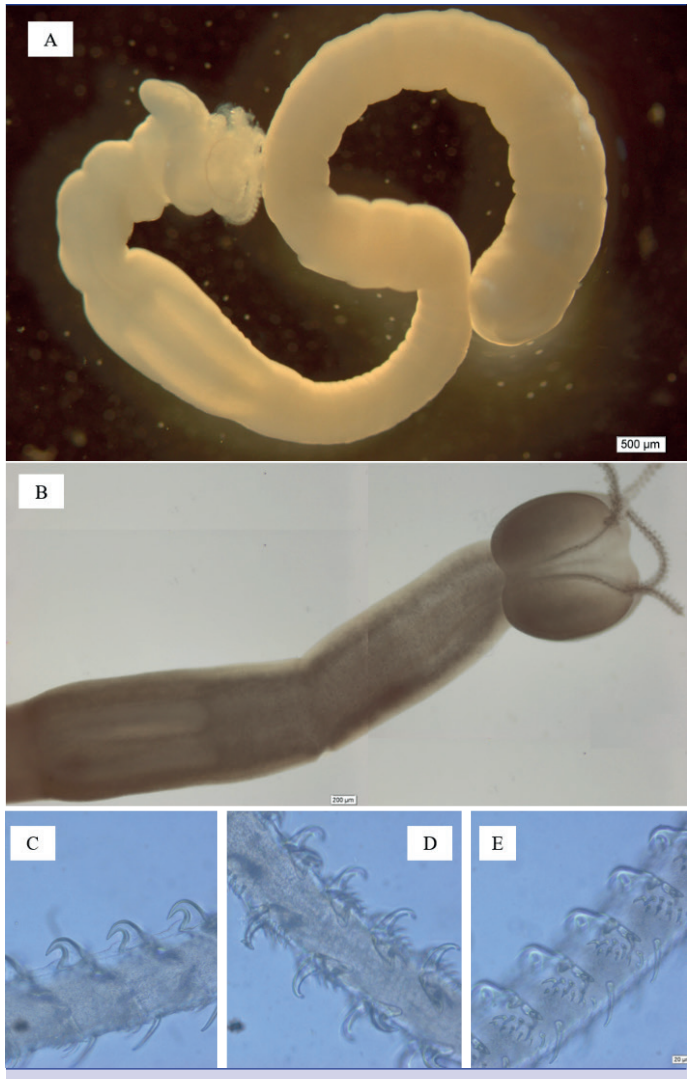

Figure 3. **A)** Mature individual of *Grillotia erinaceus* from *Raja clavata*, **B)** Scolex with four tentacular tubes and bulbs of mature cestode, **C)** Profile hook 1 tentacular tube, **D)** Profile of hook 2, **E)** Profile of hook 3 and 4.

Table 1. Measurements (mm) of *Grillotia erinaceus* (van Beneden, 1858) provided by different authors.

Measurements	Beveridge and Campbell (2007)	Kornyushin and Solonchenko (1977)	This study	This study
Host	*Raja clavata*	*Raja clavata*	*Raja clavata*	*M. merlangus*
	n=10	-	n=10	n=10
Stage	Gravid - mature	Gravid-mature	Mature	Plerocercus
Length of scolex	2.56-4.32 (3.50)	3.5-7.0	4.55-7.21 (5.96)	9.50-11.45 (10.93)
Width of scolex	0.39-0.83 (0.60)	-	1.11-1.55 (1.35)	1.90-3.15 (2.69)
Pars bothrialis	0.44-0.73 (0.60)	0.65-1.20	0.73-0.88 (0.81)	1.80-2.45 (2.18)
Pars vaginalis	1.46-2.74 (2.11)	1.30-3.70	2.22-3.34 (2.98)	5.92-7.84 (6.87)
Bulb length	0.94-1.63 (1.34)	0.90-2.00 (1.50)	0.88-1.61 (1.16)	3.18-4.35 (3.87)
Bulb width	0.16-0.25 (0.18)	0.18-0.37 (0.25)	0.18-0.23 (0.21)	0.40-0.62 (0.50)
Pars post-bulbosa	0-0.37 (0.13)	-	0.72-1.38 (1.01)	1.10-1.93 (1.56)
Hook 1 (length)	42-68 (57)	45-50	32-38 (34.65)	36.5-49.15 (43.3)
Hook 1 (base)	34-53 (45)	40-42	40-48 (42.00)	36.0-49.10 (45.3)
Hook 2 (length)	46-67 (55)	45-50	28-32 (30.50)	28.5-36.2 (33.2)
Hook 2 (base)	21-29 (26)	26	19.5-20.5 (20.05)	25.7-33.1 (28.0)
Hook 3 (length)	49-61 (54)	60	37-41 (38.70)	38.6-49-4 (43.4)
Hook 3 (base)	10-14 (13)	-	6.9-7.5 (7.06)	18.2-24.2 (21.7)
Hook 4 (length)	49-61 (54)	60	37-40 (38.10)	39.5-46.2 (43.4)
Hook 4 (base)	10-14 (13)	-	10-12 (10.55)	9.10-12.3 (10.8)

ties in the Black Sea. General features of the parasite are all in accordance with Kornyushin & Solonchenko (1978) and Beveridge & Campbell (2007) with some differences in measurement data of several parts of the scolex and tentaculate armatures as a result of possibly different environmental and host factors (Table 1).

CONCLUSION

In the present study, we provided the first comprehensive data on both the light and ultrastructural observations of *Grillotia erinaceus* plerocercoids infecting the Black Sea whiting, *Merlangius merlangus*, and adults infecting the thornback ray *R. clavata* off the Turkish coasts of the Black Sea. All the illustrations and morphometric data presented here make further contributions to our current knowledge and will also provide a base for further studies.

Acknowledgements: Authors are grateful to TUBİTAK and NASU for their financial support. Some parts of this study were previously presented as poster in an international symposium.

Ethics Committee Approval: This study was carried out in accordance with animal welfare and trial ethics. All procedures were performed in accordance with the Law on Veterinary and Medical Activities and National Animal Welfare Act.

Financial disclosure: Some parts of this study were supported financially by the Turkish Scientific and Technological Council (TÜBİTAK) in Turkey and the National Academy of Science of Ukraine (NASU) in Ukraine with the project number 110O475.

Conflict of interest: The authors declare that they have no conflicts of interest.

REFERENCES

Beveridge, I. & Campbell, R. A. (2007). Revision of the *Grillotia erinaceus* (van Beneden, 1858) species complex (Cestoda: Trypanorhyncha), with the description of *G. brayi* n.sp. *Systematic Parasitology, 68*, 1–31. [CrossRef]

Brickle, P., MacKenzie, K. & Pike, A. (2006). Variations in the parasite fauna of Patagonian toothfish (*Dissostichus eleginoides* Smith, 1898), with length, season and depth of habitat around the Falkland Island. *Journal of Parasitology, 92*(2), 282–291. [CrossRef]

Chubb, J. C., Pool, D. W, & Veltkamp, C. J. (1987). A key to the species of cestodes (tapeworms) parasitic in British and Irish freshwater fishes. *Journal of Fish Biology, 31*, 517-543. [CrossRef]

Deardorff, T. L., Raybourne, R. B. & Mattis, T. E. (1984). Infections with trypanorhynch plerocerci (Cestoda) in Hawaiian fishes of commercial importance. *Sea Grant Quarterly, 6*, 1-6.

Dollfus, R. P. (1942). Études critiques sur les Tétrarhynques du Museum de Paris. *Archives du Muséum national d´ Histoire natürelle, 19*, 1–466.

Jones, M. K., Beveridge, I., Campbell, R. A. & Palm, H. (2004). Terminology of the sucker-like organs of the scolex of trypanorhynch cestodes. *Systematic Parasitology, 59*, 121–126. [CrossRef]

Kornyushin, V. V. & Solonchenko A. I. (1978). Redescription of the cestodes *Grillotia erinaceus* (Beneden, 1858) and *Christianella minuta* (Beneden, 1849) from Chondrostei in the Black Sea. *Biologiya Morya, 45*, 26–33 (in Russian).

Lubieniecki, B. (1976). Aspects of the biology of the plerocercoid of *Grillotia erinaceus* (van Beneden, 1858) (Cestoda: Trypanorhyncha) in haddock *Melanogrammus aeglefinus* (L.). *Journal of Fish Biology, 8*, 431–439. [CrossRef]

Menoret, A. & Ivanov, V. A. (2012). Description of plerocerci and adults of a new species of *Grillotia* (Cestoda: Trypanorhyncha) in teleosts and elasmobranchs from the Patagonian shelf off Argentina. *Journal of Parasitology, 98*(6), 1185-1199. [CrossRef]

Özer, A., Öztürk, T., Kornyushin V., Kornyychuk, Y. & Yurakhno, V., (2012). Light and Scanning Electron Microscopic Observations on *Grillotia erinaceus* (van Beneden, 1858) (Cestoda:Trypanorhyncha) plerocercoids in whiting, *Merlangius merlangus* (L., 1758). XI. European Multicolloquium of Parasitology (EMOP XI), 24-29 July 2012, Cluj-Napoca, Romania.

Özer, A., Öztürk, T., Kornyushin, V., Kornyychuk, Y. & Yurakhno, V. (2014). *Grillotia erinaceus* (van Beneden, 1858) (Cestoda: Trypanorhyncha) from whiting in the Black Sea, with observations on seasonality and host-parasite interrelationship. *Acta Parasitologica, 59*(3), 420-425. [CrossRef]

Palm, H. W. (2004). The Trypanorhyncha Diesing, 1863. PKSPL-IPB Press, Bogor, x+710 pp

Palm, H. W. & Caira, J. N. (2008). Host specificity of adult versus larval cestodes of the elasmobranch tapeworm order Trypanorhyncha. *International Journal for Parasitology, 38*, 381–388. [CrossRef]

Palm, H. W. & Klimpel, S. (2007). Evolution of the parasitic life in the Ocean. *Trends in Parasitology, 23,* 10–12. [CrossRef]

Palm, H. W., Waeschenbach, A., Olson, P. D. & Littlewood, D. T. J. (2009). Molecular phylogeny and evolution of the Trypanorhyncha Diesing, 1863 (Platyhelminthes: Cestoda). *Molecular Phylogenetics and Evolution, 52*, 351–367. [CrossRef]

Palm, H. W., Yulianto, I. & Piatkowski, U. (2017). Trypanorhynch Assemblages Indicate Ecological and Phylogenetical Attributes of Their Elasmobranch Final Hosts. *Fishes, 2*(8), 1-16. [CrossRef]

Shively, S, & Miller, W. R. (2009). The use of HMDS (hexamethyldisilazane) to replace Critical Point Drying (CPD) in the preparation of tardigrades for SEM (Scanning Electron Microscope) Imaging. *Transactions of the Kansas Academy of Science, 112 (3-4),* 198–200. [CrossRef]

Tepe, Y., Oğuz, M. C. & Heckmann, R. A. (2014). Digenean and cestode parasites of teleost fish from the Eastern Black Sea Region. *Turkish Journal of Zoology, 38*, 209-215. [CrossRef]