International Journal of Research in Engineering, Science and Management
Volume-3, Issue-3, March-2020
www.ijresm.com | ISSN (Online): 2581-5792

A New Species of the Genus *Proteocephalus* (Eucestoda: Proteocephalidea: Proteocephalinae) Parasitizing *Wallago Attu* in Ropar Wetland, Punjab, India

Deepika Jasrotia[1], Harpreet Kaur[2]
[1]*Research Scholar, Department of Zoology and Environmental Sciences, Punjabi University, Patiala, India*
[2]*Associate Professor, Department of Zoology and Environmental Sciences, Punjabi University, Patiala, India*

***Abstract*: *Proteocephalus wallagoi* n. sp. (Cestoda: Proteocephalidea), specimen were collected from the small intestine of a Indian freshwater catfish *Wallago attu* from Ropar wetland in Punjab, India. The present species was characterized in having rectangular proglottids measure 779-781×2114-2116µm in size, testes 200-240 in number, ovary bilobed with highly coiled oviduct. Vagina opens posterior to the pyriform shaped cirrus sac. Vitellaria follicular located in the medullary region occupy whole of the lateral margins of mature proglottid.**

***Keywords*: Cestode, *Proteocephalus* species, Ropar wetland, Siluroid fishes, *Wallago attu*.**

1. Introduction

The large number of species places in the genus *Proteocephalus* Weinland, 1858 with numerous taxa occurring in the Palaearctic region (Freze 1965, Priemer 1982, Schmidt 1986, Chubb *et al*. 1987, Dubinina 1987, Scholz 1989, Škeříková *et al*. 2001, Scholz *et al*. 2007). Proteocephalidean parasites are mostly present in fishes, amphibians and reptiles. In the present study cestode worms belonging to the genus *Proteocephalus* were most frequently encountered in the small intestine of *Wallago attu*.

These tapeworms have been reported to exhibit great intraspecific variations parasitic in various siluroid hosts. These worms have distinct external segmentation, one set of gonads per segment, numerous testes, a bilobed ovary at the posterior end of the proglottid, follicular and lateral vitellaria, a lateral genital pore, and a scolex with four sucker and an apical sucker may be present. The identification of *Proteocephalus* species is often difficult due to a general uniformity in their strobilar morphology (Freze, 1965). Brabec *et al*. 2015 and Chambrier *et al*. 2015 have demonstrated limitation of morphological characters of proteocephalidean and bothriocephalidean cestodes respectively. Since there is a limited data of proteocephalidean cestodes from Indian catfish, this work has been undertaken to characterized these highly polymorphic species having enoromous morphologically variations. In the present study a new species of the genus *Proteocephalus* has been described based on the assessment of morphological characters of scolex and strobila after close comparsion with already known species.

2. Material and Methods

The fresh specimens of the catfish, *Wallago attu* vern. mullee were collected from the local fish market near Ropar wetland, Punjab and were brought to the laboratory for parasitological examination. The worms were gently removed from the gut lining and placed in a petridish with physiological saline (0.8% NaCl). 3-4 worms were detected per intestine. Each worm was relaxed and stretched in warm 4% formalin over the edge of the beaker, fixed and preserved in fresh 4% formalin for the preparation of whole mounts. The Gower's carmine was used to stained worm, differentiated in 70% acid ethanol, dehydrated through an ethanol series and mounted in DPX. Measurements were taken with the help of calibrated micrometer (µm) or otherwise mentioned. Line drawings were made from stained material with the help of camera lucida. The identification of the tapeworms at the generic level was done with the help of "Keys to the cestode parasites of vertebrates" by (Khalil et al. 1994).

3. Results

Description of present species is based on 3 specimens,

General morphology: Parasites 209mm in length. Scolex 749-751×524-526µm in size, globular, unarmed distinctly separated from neck, apical sucker distinct 164-166×239-241µm. Suckers four, lateral in position, measure 164-166×209-211µm. Neck 584-586µm width.

Ratio: Diameter of apical sucker to diameter of sucker (DAS/DS): 68.7%. Diameter of apical sucker to its length (DAS/LAS): 68.7%. Diameter of sucker diameter to scolex width (DS/WS): 31.42%. Diameter of apical sucker to width of scolex (DAS/WS): 45.7%.

Strobila: Anterior immature proglottid broader than long 299-30×1424-1426 µm, mature proglottid rectangular in shape,

measure 779-781×2114-2116 µm in size.

Reproductive organs: Testes small, rounded and 200-240 in number, each lobule 44-46×59-61µm in size occupying whole of space above the ovary. Testes in single file in the medullary parenchyma (observed in transverse section Fig. 3a). Ovary large, bilobed butterfly-shaped, measure 254-256×974-976µm in size and situated at the posterior margin of the proglottid. Oviduct arise from the middle of the ovary. Oviduct highly coiled spanning one side of the proglottid. Vagina open posterior to the cirrus pouch. Cirrus pouch pyriform 119-121×194-196µm in size, cirrus sac short cover less than 9.2% width of the proglottid, vas deferens highly coiled, reaching to midline of proglottid. Genital pore is oval in shape and irregularly alternate, at the centre of the lateral margin of proglottid. Vitellaria follicular, oval to rounded shaped, follicles occupying lateral margin of the whole length of the mature proglottid (Fig. 1 a-c, Fig. 2 a-d).

Taxonomic summary of *Proteocephalus wallagoi* n. sp.

Host: Wallago attu (Bloch and Schneider) vern. mullee

Type locality: Ropar Wetland, Punjab

Site of infection: Small Intestine

Etymology: The specific epithet "*wallagoi"* has been given after the generic name of the host fish.

Type specimen: Cestodes worm stained in Gower's carmine, deposited in Parasitology Laboratory in the Department of Zoology, Panjab University, Chandigarh, India Slide no: SN1 – WA/ P/2015-16.

Histology of mature proglottid: Testes oval, forming a single layer in the medullary region. Vitelline follicles medullary laterally placed in the proglottid (Fig. 3 a-d).

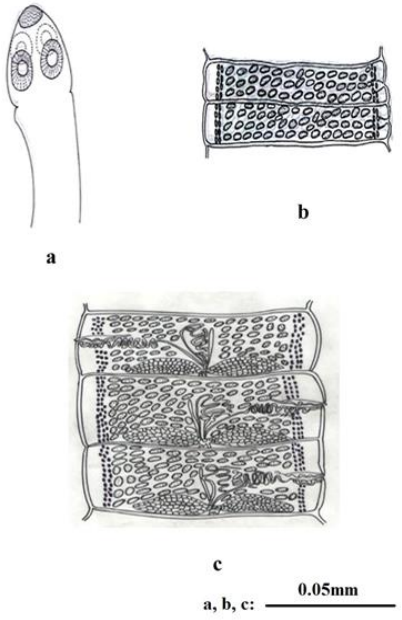

Fig. 1. Line drawing of *P. wallagoi* n. sp. a) Scolex, b) Immature proglottid, c) Mature proglottids

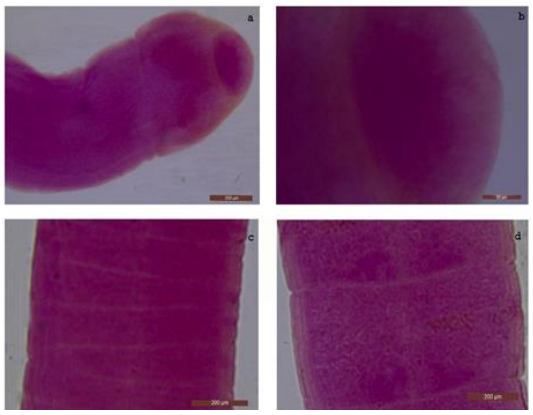

Fig. 2. Photomicrograph of *P. wallagoi* n. sp. a) Scolex, b) Apical sucker, c) Immature proglottid, d) Mature proglottid stained with Gower's carmine

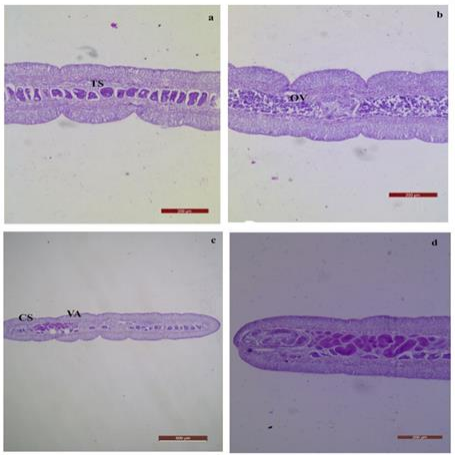

Fig. 3. a) T.S. of mature proglottid showing single layer of testes, b) T.S. of mature proglottid showing ovary, c) T.S. of mature proglottid showing cirrus sac (CS) and vas deferens (VA), d) Magnifying view of cirrus sac and vas deferens stained with haematoxylin-eosin

4. Discussion and Conclusion

The scolex of the present specimen of the genus *Proteocephalus* was compared with that of *P. osculates* (Goeze, 1782); *P. thymalli* (Annenkova-Chlopina, 1923); *P. macrocephalus* (Creplin, 1825); *P. pollanicola* Gresson, 1952; *P. percae* (Muller, 1780); *P. cernuae* (Gmelin, 1790); *P. exiguus* La Rue, 1911 and *P. vazzolerae* (Pavanelli and Takemoto, 1995) in having an apical sucker. In this character the present species was closer to *P. percae, P. cernuae and P. exiguus* in which the apical sucker was flattened and differ from *P. osculatus, P. thymalli, P. macrocephalus* and *P. pollanicola* in having high apical sucker. According to Scholz *et al*. (1998) high intraspecfic variability in this feature and the coefficient of variability reached to maximum value (25 to 30%) in *P. pollanicola, P. exiguus* and *P. thymalli*. Furthermore, in the present species as regards to the ratio of apical sucker diameter to length (DAS/LAS) it was 68.7% wider than long in *P. macrocephalus, P. osculatus* and in the rest of other species it was more flattened. The apical sucker showed high intraspecific variability and the coefficient of variability reached to

Table 1
Comparative description of *P. wallagoi* n. sp. with original description of morphologically closely related other species

Name of the parasite species → / Characteristics ▼	***P. vitellaris* Verma, 1928**	***P. ritaii* Verma, 1926**	***P. sophiae* de Chambrier and Rego, 1994**	***P. wallagoi* n. sp. Present study**
Body length	250mm	125mm	-	209mm
Scolex size	520×800	144×224	525-670 in diameter	749-751×524-526
Apical organ (Size)	-	-	120-165	164-166 × 239-241
Suckers (Size)	160 in diameter	45	255-355	164-166
Proglottid (Size) **Anterior** **Mature** **Gravid**	 - - -	 - -	 - -	 299-301 × 1424-1426 779-781 × 2114-2116
Testes **Number** **Size**	 250-275 -	 150-200 40-60 diameter	 83-141	 200-240 44-46 × 59-61
Ovary	-	-	530-710 wide 200-620 long	254-256 × 974-976
Cirrus pouch	140 in width	192 long 128 wide	300-410 long 100-145 wide	119-121 × 194-196
CS/ WP	-	10-14%	-	9.2%
WP/ VA	-	-	-	45%
Number of uterine diverticula	5	8-12	35-45	-
Position of vagina relative to cirrus pouch	-	-	-	Posterior
Host	*Bagarius yarellis*	*Rita rita*	*Paulicea luetkeai*	*Wallago attu*
Location	Small Intestine	Small Intestine	Small Intestine	Small Intestine
Locality	Allahabad, India	Allahabad, India	Brazil	Ropar wetland,Punjab, India

All measurements are in micrometer unless otherwise mentioned.

Table 2
Comparative description of scolex of *P. wallagoi* n. sp. with scolices other closely related species from Palaeractic region

Name of the parasite species ▶ / Characteristics ▼	***P. macrocephalus* (Creplin, 1825)**	***P. osculates* (Goeze, 1782)**	***P. exiguus* La Rue, 1911**	***P. percae* (Muller, 1780)**	***P. cernuae* (Gmelin, 1790)**	***P. pollanicola* Gresson, 1952**	***P. thymalli* (Annenkova-Chlopina, 1923)**	***P. wallagoi* n. sp. (Present study)**
Width of scolex	272-484	352-440	134-205	141-214	288-368	202-304	376-780	524-526
Width of neck	128-272	232-368	-	-	304-360	202-246	224-480	584-586
Diameter of sucker	72-116	108-139	46-72	49-64	75-108	86-122	147-227	164-166
Diameter of apical sucker	29-42	64-86	-	23-37	28-55	52-90	58-96	239-241
Length of apical sucker	26-48	58-90	13-31	14-23	18-23	30	26-64	164-166
DS/WS	0.21-0.32	0.29-0.38	0.24-0.40	0.23-0.39	0.21-0.31	0.31-0.46	0.24-0.43	0.30-0.32
DAS/LAS	0.76-1.23	0.71-1.43	0.70-2.38	1.22-2.43	1.33-2.39	1.82-3.35	1.24-2.66	1.44-1.46
DAS/DS	0.29-0.48	0.48-0.73	0.36-0.81	0.38-0.73	0.33-0.51	0.56-0.90	0.35-0.44	1.44-1.46
Host	Eels *(Anguilla anguilla*)	*Silurus glanis*	Trout Salmo *trutta trutta*	*Perca fluviatilis*	Ruff *Gymnocephalus cernuus*	*Coregonus pollan*	Grayling *(Thymallus thymallus)*	*W. attu*
Locality	Germany	Czech Republic	Germany	Denmark	Germany	UK	Lake Baikal, Russia.	Ropar wetland, Punjab, India

All measurements are in micrometer unless otherwise mentioned.

maximum value (25 to 30%) in *P. pollanicola*, *P. exiguus* and *P. thymalli*.

The ratio of diameter of apical sucker to diameter of lateral suckers on the scolex (DAS/DS) indicated relatively large apical sucker in the present species, presenting about 68% diameter of suckers. In this respect, the present specimen was closer to *P. exiguus* in having 42-75% of the diameter of lateral suckers. However, differ from *P. macrocephalus*, *P. cernuae* which have smaller apical sucker representing less than 50% of the diameter of lateral suckers, also from *P. percae*, *P. exiguus*, *P. osculatus and P. pollanicola* in having very large apical sucker with diameter 90% of the lateral suckers (Scholz *et al*., 1998).

Ratio of diameter of apical sucker to width of the scolex DAS/WS of the present species was different from all other species as apical sucker represented 46% i.e less than half of the scolex width. This character was different from *P. thymalli*, *P. percae*, *P. exiguus and P. osculatus* in which DAS/WS was more than 1/4 of scolex width i.e about 10%. Scolex width indicates relatively small apical sucker (Scholz *et al*., 1998). The present species was distinguished from *P. sophiae* (Chambrier and Rego, 1994) and *P. chubbi* (Pavanelli and Takemoto, 1995) as the apical sucker was absent. In the present species, the vitellaria was medullary and occupied the lateral margins along the total length of the proglottid, therefore distinguished from *P. sophiae* infecting siluroid fish from the Brazilian Amazon and *P. macdonaghi* infecting *Basilichthys microlepidotus*, Argentina, in lacking the preporal vitellaria and apical sucker in the later.

The present species was closely compared with two other Indian species i.e. *P. ritaii* (Verma, 1926) and *P. vitellaris* (Verma, 1928) Southwell,1930. *P. ritaii* differ from the present species in having an apical sucker in the form of a mere muscle plug as a remnant of a muscular rostellum and peculiar longer unsegmented neck, shorter worm length (209µm vs 125µm); number of testes (200-240 vs 150-200) and longer scolex (749-751µm vs 144µm). As for *P. vitellaris*, the present species was different in having scolex longer than wide (749-751µm vs 520µm); the proglottid size much larger than the present species (779-781×2114-2116 µm); suckers (164-166µm vs 160µm); bilobed and larger size of ovaries 254-256 × 974-976µm and number of testes (200-240 vs 250-275), single layer of testes as seen (Fig.3a), cirrus sac shorter covering less than 9.2% width of the proglottid, vas deferens highly coiled, covering 45% reaching to width of proglottid.

Therefore, the present species, *P. wallogoi* n. sp. differ from all the above mentioned species from the characters which include, scolex morphology, size of proglottid, strobila length, diameter of suckers, number of testes and size of ovary. In view of the above differences, the present species under study is proposed as new to the science and named as *P. wallagoi* n. sp.0b

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