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A new species of genus *Paryphostomum* Dietz, 1909 (Trematoda: Echinostomatidae) in *Phalacrocorax niger* of Pakistan

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Abstract
During examination of Little Cormorant *Phalacrocorax niger* for the presence of helminth parasites, a total of 105 trematodes belonging to genus *Paryphostomum* Dietz, 1909 were recovered from intestine of 5 hosts. These trematodes have large, elongated body, reniform head collar equipped with 22 spines, large acetabulum, oval cirrus sac, anterior testis tri-lobed, spherical ovary, broad uterus and prominent lateral vitellaria. These were compared with closely related species and found differing from their congeners in number and size of collar spines, acetabulum, uterus and eggs, shape of cirrus sac, position of ovary and position and shape of testes. On the basis of these diagnostic differences, a new species *Paryphostomum sanghari* is proposed to accommodate the present worm. Species name refers to the locality of host bird.

Keywords: Avian trematode, *Paryphostomum sanghari* new species, little cormorant, Sindh, Pakistan

Introduction
The genus *Paryphostomum* Dietz, 1909 contains common intestinal parasites of cormorants, water turkey, ducks and snipe. It is particularly reported in various birds including *Tringa*, *Anhinga*, *Rallus*, *Phalacrocorax*, *Podiceps*, *Anser*, *Bubulcus*, *Oenopus*, *Coragyps*, *Capella*, *Gallinula*, *Gallus*, *Dendrocygna* and *Porphyrio* species of Europe, Africa, America, Asia and Australia [1, 2]. Several workers have reported many species of this genus. Though the birds are definitive hosts for *Paryphostomum* species, however, a rare infection of *Paryphostomum sufrartyfex* has been noticed in human [3]. Present species of *Paryphostomum* is recovered from intestine of Little Cormorant *Phalacrocorax niger* which is migratory as well as resident bird in Pakistan. It is a gregarious mainly piscivorous in habit some time forage singly or in loose group in ponds, lakes, streams and coastal areas [4, 5, 6]. Little Cormorant is commonly found in District Sanghar Sindh Pakistan [7]. Moreover, reports are available on helminth parasites of *Phalacrocorax niger* in Pakistan includes: Akram [8, 9]. Previously, there is no report on the genus *Paryphostomum* in Pakistan.

Material and methods
Eleven little cormorants were caught alive during December 2015 from District Sanghar of Sindh Pakistan and brought in Parasitology laboratory of Department of Zoology, University of Sindh, Jamshoro. Host birds were chloroformed and dissected. The visceral organs were removed and examined on stereomicroscope for the presence of helminth parasites. The trematodes collected were fixed in 70% ethanol, stained with borax carmine, dehydrated in graded alcohol series, cleared in clove oil and Xylol. Specimens were permanently mounted in Canada balsam. The drawing lines were made with camera Lucida and photographs taken with Nikon digital camera. The measurements were taken in millimeter (mm). The identification of specimens was made accordance to keys given by Jones *et al*. [10]; Yamaguti [2] and relevant literature**.**

Results
During present study eleven Little Cormorants were examined and five were infected with 105 *Paryphostomum* specimens. These were recovered from intestine and their infection rate was 45.45%.

Description

Body large, elongated, measuring 5.72-5.12 mm long and 0.88-0.76 mm wide. Maximum width at testicular level. Fore-body short, measuring 0.909-0.709 and hind-body elongated, measuring 3.939-3.139 in size. Head collar well developed, reniform, measuring 0.34-0.24 long and 0.4-0.20 wide, equipped with 22 spines. Corner spines (2 X 3) pointed, larger than marginal spines (2 X 8), measuring 0.118-0.112 in length and 0.04-0.03 in width. Oral sucker small, spherical, subterminal, measuring 0.14-0.12 in diameter. Pre-pharynx short. Pharynx muscular, elongated, larger than pre-pharynx, measuring 0.24-0.21 long and 0.14-0.10 wide. Esophagus long, measuring 0.48-0.43 bifurcated into ceca in front of ventral sucker. Ventral sucker prominent strongly muscular cup-shaped, situated in first quarter of body, measuring 0.72-0.69 long and 0.68-0.62 wide. Cirrus pouch oval, measuring 0.28-0.22 long and 0.16-0.12 wide located in between anterior margins of ventral sucker and cecal fork. Testes lobed, tandem, median, post-acetabular, located in middle of hindbody. Anterior testis tri-lobed, situated in second quarter of hindbody, measuring 0.64-0.60 long and 0.52-0.49 wide. Posterior testis multilobed (two prominent and four smaller lobes) situated in beginning of third quarter of hindbody, measuring 0.64-0.61 long 0.52-0.46 wide. Post-testicular area densely filled with vitelline, measuring 1.5-1.2 long. Genital pore situated anterior of ventral sucker and behind bifurcation of ceca.

Ovary spherical, median, pre-testicular, post-acetabular situated near anterior testis than ventral sucker in end of first quarter of hindbody, measuring 0.32-0.29 long and 0.36-0.31 wide. Seminal receptacle oval, situated at left corner of ovary. Uterus pre-testicular, post-acetabular, not coiled, broad with eggs, situated in between ventral sucker and ovary. Eggs not numerous, measuring 0.0814-0.0714 long and 0.066-0.054 wide. Vitellaria follicular, present in lateral sides of body, starting from lower level of ventral sucker extending up to posterior end of body. Excretory pore terminal. Excretory vesicle Y-shaped

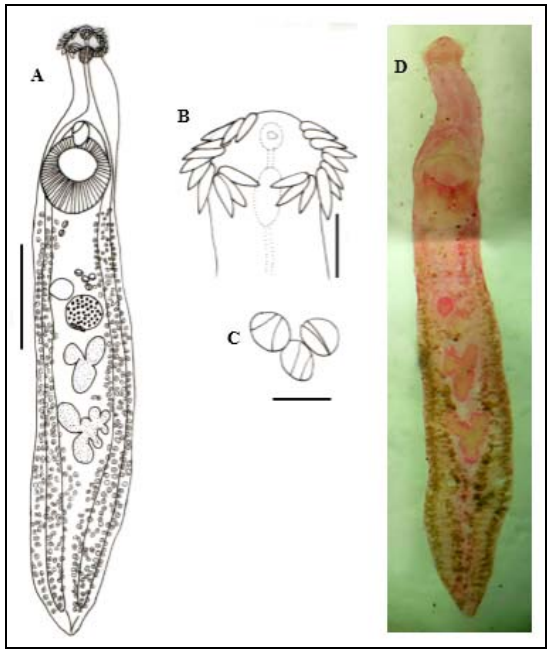

Fig 1: *Paryphostomum sanghari* n.sp. A. Entire worm; B. Head collar and C. Eggs. D. Photograph of entire specimen. ***Scale bar*:** A. 1 mm; B. 0.2 mm and C. 0.1 mm

Table 1: Comparison of various species of genus *Paryphostomum*

Name of organ	*P. sanghari* n.sp.	*P. radiatum*	*P. giganticum*	*P. testitrifoluim*	*P. segregatum*
Body	Elongated (5.7-5.12 X 0.76-0.88)	Elongated (5.10 X 0.97)	Elongated (7.69 X 1.96)	Elongated to oval (4.0-5.44 X 0.57-0.84)	Elongated (3.24-3.34 X 0.55-0.76)
Head collar	Reniform (0.24-0.34 X 0.20-0.40)	Reniform	Reniform	Reniform (0.36-0.52)	Reniform (0.17-0.31 X 0.33-0.43)
Spines	22	27	47	27	27
Oral sucker	Sub-terminal (0.14)	Sub-terminal (0.16 X 0.17)	Sub-terminal	Sub-terminal, spherical (0.01-0.2 X 0.11-0.17)	Sub-terminal, spherical (0.11-0.13 X 0.12-0.18)
Ventral Sucker	Large, round (0.72-0.69 X 0.62-0.68)	Large, round (0.67 X 0.6)	Large, round	Muscular, cup-shaped (0.51-0.72)	Cup-shaped (0.38-0.44 X 37-46)
Pharynx	Muscular (0.21-0.24 X 0.10-0.14)	Muscular (0.18 X 0.14)	Muscular	Sub-globular (0.16-0.28 X 0.09-0.20)	Sub-globular (0.12-0.14 X 0.09-0.10)
Esophagus	Bifurcated into ceca in front of acetabulum	Bifurcated into ceca in front of acetabulum	Bifurcated into ceca in front of acetabulum	Short, bifurcated anterior to acetabulum (0.32-0.44)	0.35-0.52
Ceca	Extended from acetabulum to posterior end	Extended from acetabulum to posterior end	Extended from acetabulum to posterior end	Extended from acetabulum to posterior end	Extended from acetabulum to posterior end
Testes	Tandem, sub-median. Anterior testis trilobed (0.64-0.6 X 0.52-0.49). Posterior testis large, multilobed (0.64-0.61 X 0.46-0.52)	Tandem, median, deeply lobed. Anterior testis (0.49 X 0.45). Posterior testis (0.574 X 0.468)	Slightly lobed, situated in posterior of forebody	Tandem, median, tri-lobed. Anterior testis equatorial (0.25-0.40 X 0.10-0.34). Posterior testis middle of hindbody (0.32-0.56 X 0.12-0.34)	Tandem, deeply lobed, contiguous. Anterior testis (0.26-0.46 X 0.37-0.64). Posterior testis (0.42-0.60 X 0.33-0.58)
Ovary	Round (0.29-0.32 X 0.31-0.36)	Oval (0.13 X 0.27)	Oval	Small, spherical, pre-testicular (0.09-0.19 X 0.72-0.16)	Small, elongated-oval (0.13 0.19 X 0.14-0.19)
Cirrus sac	Oval, pre-acetabular	Oval, pre-acetabular	Oval, pre-acetabular	Elongated-oval	Elongated-oval, mid of acetabulum
Uterus	Broad, with eggs	Coiled filled with eggs	Coiled filled with eggs	Short, not much coiled	Short

Eggs	0.08-0.07 X 0.06-0.05	0.09 X 0.05	---	0.04-0.09 X 0.03-0.06	0.07-0.10 X 0.04-0.06
Vitellaria	Follicular	Follicular	Follicular	Follicular	Follicular
Host	*Phalacrocorax niger*	*Phalacrocorax carbo*	*Gallinula chloropus*	*Phalacrocorax carbo, Phalacrocorax pygmaeus*	*Coragypys atratus*
Location	Intestine	Intestine	Intestine	Intestine	Intestine
Locality	Sanghar, Pakistan	Czeck Republic	India	Iraq and India	Paraguay

Taxonomic summary

Host	Little cormorant
No. of specimens recovered:	105
No. of hosts found positive:	05
Site of infection:	Intestine
Locality:	Sanghar, Sindh, Pakistan
Etymology:	District's name Sanghar from where the hosts were collected

Discussion

Genus *Paryphostomum* Dietz, 1909 includes flukes collected from avian hosts. *P. radiatum* (Dujardin, 1845) Dietz, 1909 is the type species of this genus. Different researchers have explored various species of this genus [11]. Several species of the genus include *Paryphostomum agrawali* Gupta and Singh, 1986; *P. baiyangdiensis* Ku *et al.*, 1973; *P. bubulcusi* Agarwal, 1959 [12]; *P. carbonis* Mendheim 1940; *P. dollfusi* Agarwal 1959; *P. durgensis* Sapre 1969; *P. fragosum* Dietz, 1909; *P. giganticum* Rai and Agarwal, 1961; *P. globorchum* Oshmarin, 1971; *P horai* Baugh, 1950; *P. huaccaci* Ibanez, 1974; *P. indicum* Bhalerao, 1931; *P. jaini*; *P. lobulatum* Odhner, 1910; *P. mehrai* Faruqui; *P. mexicanum* Lamothe-Argumedo and Leon, 1989; *P. mukhtarensis* Ahmed, 2000; *P. neotoma* Jain, 1953; *P. novum* Verma, 1936; *P. palustris rattus* Jain, 1953; *P. parvicephalum* Rietschel and Werding, 1978; *P. pentalobum* Verma, 1936; *P. phalacrocoracis* Gross 1941; *P. radiatum* (Dujardin, 1845) Dietz, 1909; *P. sarcorhamphus*; *P. segregatum* Dietz, 1909; *P. siddiqui* Gupta and Singh, 1986; *P. srivastavi* Ahmed, 2000; *P. sufrartyfex* Lane, 1915; *P. tenuicollis*; *P. testitrifolium* Gogate, 1934; *P. udaipurensis*, Sharma, 1976.

P. dolfusi Agarwal, 1959 reported from *Bubulcus ibis* of India differs from present species in body size (11.08-13.36 X 1.95-2.52), number of collar spines (46). *P. giganticum* Rai and Agarwal, 1961[13] reported from *Bubulcus ibis* and *Porphyrio poliocephalus* from India differs from present species in having largest body size (16.15-18.59 X 1.94), number of collar spines (47), slightly lobed testes located in posterior half of body, ovary oval and coiled uterus. *P. horai* Baugh, 1950 reported from *Anas poecilorhyncha* of India differs from present species in having averagely smaller body size (3.05-6.97 X 0.61-1.72), number of collar spines (38-39) and multilobed testes. *P. lobulatum* Odhner, 1910 reported from *Phalacrocorax africanus* of White Nile differs from present species in having smaller body size and shape (2.25 X 1.25), number of collar spines (27) and numerous lobes of testes. *P. novum* Verma, 1936 reported from ducks and geese of India and Azerbaidzhan differs from present species in large body size (9.7-10.8 X 1.15-2.44), number of collar spines (37) and lobed testes. *P. segregatum* Dietz, 1909 reported from *Cathartes*, *Sarcorhamphus*, *Catharista*, *Oenops* and *Coragyps* species of Brazil, Venezuela and Paraguay differs from present species in having smaller body size (3.243-3.348 X 0.559-0.769), head collar (0.175-0.315 X 0.336-0.433), oral sucker (0.112-0.133 X 0.119-0.182) and ventral sucker (0.384-0.440 X 0.370-0.468), shape and size of testes deeply lobed, shape of testes and size of anterior testis (0.259-0.461 X 0.377-0.643), posterior testis (0.419-0.601 X 0.336-0.587) and elongated oval shape and location of cirrus sac in mid of ventral sucker. *P. radiatum* (Duj., 1845) Dietz, 1909 [14] reported from *Phalacrocorax*, *Podiceps*, *Anhinga* and *Anser* of Srilanka, Russia, Azerbaidzhan and Japan differs from present species in having slightly smaller body (5.10 X 0.97), number of collar spines (27), slightly large oral sucker (0.165 X 0.171), smaller pharynx (0.179 X 0.139), ventral sucker (0.677 X 0.59) and ovary (0.138 X 0.271) multilobed testes, coiled uterus and smaller eggs (0.091 X 0.058). *P. testitrifoluim* Gogate, 1934 [15] reported from *Dendrocygna javanica, Phalacrocorax pygmaeus* and *P. carbo* of Russia and Rangoon differs from present species in having smaller body size (4.0-5.44 X 0.574-0.840), elongated-oval body shape, number of collar spines (27), slightly coiled uterus, shape of testes and smaller eggs (0.040-0.096 X 0.032-0.064). *P. mukhtarensis* Ahmed, 2000[16] reported from *Phalacrocorax carbo sinesis* of Kashmir differs from present species in having smaller body size (5.5 X 0.739), dorsoventrally flattened body shape, number of collar spines (26), pre-pharynx absent, testes post-equatorial, pear-shaped cirrus sac, slightly coiled uterus and confluent vitellaria. *P. srivastavi* Ahmed, 2000 reported from *Phalacrocorax carbo sinesis* of Kashmir differs from present species in having broad posterior end, number of collar spines (25), post-equatorial multilobed testes, submedian ovary and coiled uterus.

Present species differs from other species of the genus *Paryphostomum* in number of collar spines, size of ventral sucker, size and number of lobes of testes, size of ovary, shape of uterus and eggs. Therefore, present specimen is recorded as a new species and named *Paryphostomum sanghari*. The name of species refers to name of district from where host was collected. However, this genus is being reported for the first time from Pakistan.

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