

ECHINOSTOMA PAKISTANENSIS SP. N. (TREMATODA: ECHINOSTOMATIDAE POCHE, 1926) FOUND IN PHALACROCORAX FUSCICOLLIS IN LARKANA, SINDH, PAKISTAN

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ABSTRACT

Echinostoma pakistanensis sp. n. was extracted from the intestine of *Phalacrocorax fuscicollis* (Indian cormorant) from Larkana, Sindh. The new species have different specific characteristics; elongated body 16.67-18.33, head collar bears dorsally uninterrupted crown of 44 spines, oral sucker is terminal, rounded 0.31-0.33 x 0.19-0.22, pre-pharynx absent, pharynx is oval 0.55-0.65 x 0.45-0.54, esophagus moderately 0.91-0.92, testes multilobed, tandem. Anterior testis smaller 1.32 –1.42 (1.36) x 0.94–0.98 (0.95), posterior testis larger 1.48 –1.49 (1.48) x 0.77–0.78 (0.77), cirrus sac is rounded, vitellaria starts from the posterior end of acetabulum and reaches to the end of excretory vesicles, eggs oval, thin walled 73.3-80.2 x 98.3-113.2.

Key words: *Echinostoma pakistanensis* sp. n. Echinostomatidae, *Phalacrocorax fuscicollis*, Larkana, Sindh, Pakistan

INTRODUCTION

Phalacrocorax fuscicollis (Stephen, 1806) belongs to family Phalacrocoracidae (Reichenbach, 1850). They are generally known as black –faced due to color of their neck and body. They are mostly found in Asia and Oceania continent and live near the coasts of river and lakes, wetlands, lagoons and ponds. The Indian cormorant primarily feeds on fish, but also eats eel and octopus. A few flukes of the genus *Echinostoma* (Rudolphi, 1809) have been reported from the same host *Phalacrocorax fuscicollis* across the world and only one *Echinostoma valentine* (Das and Ghazi, 2011) from Karachi, Pakistan. *Echinostoma pakistanensis* sp. n. discovered in the same host's intestine from a new locality, Larkana, Sindh, Pakistan.

MATERIALS AND METHODS

Seven *Phalacrocorax fuscicollis* Stephen, 1806 were shot down and trapped using nets from different areas of district Larkana from December, 2022 to March, 2023. The birds were autopsied and removed visceral organs were kept separately in petri dishes for examination of helminths. During analysis nine mature, egg bearing flukes were extracted from the small intestine. The flukes were dipped in warm 70 % ethanol to die and expand. Later worms were transferred between slides, knotted with thread and placed in solution of Formalin Acetic Acid for a day. Mayer's carmalum was used to stain specimens and dehydrated series of ethanol used for cleaning. Later specimens passed through clove oil and xylene for clearing and permanent slides made with Canada balsam. Measurements of entire specimen and different organs were taken in mm. Camera Lucida attached with light microscope was used to draw sketches. Specimens are in custody of Dr. Sanjota Nirmal Das, co-author and Parasitological sectional head at Department of Zoology, University of Sindh, Jamshoro.

RESULTS

***Echinostoma pakistanensis* sp. n
(Figs A-C)**

Host:	<i>Phalacrocorax fuscicollis</i> Stephen, 1806 (Indian cormorant)
locality:	Larkana, Sindh, Pakistan.
location:	Small Intestine
Hosts examined / infected:	7/4
specimens recovered:	9
Percentage of infection	57 %

DESCRIPTION: (BASED UPON NINE MATURE, EGGS BEARING PERMANENTLY MOUNTED FLUKES)

Body elongated, anterior part narrower than posterior measuring 16.67–18.33 (17.58) x 3.28–3.76 (3.51), maximum width is ascribed to anterior testis.

Head collar well developed, measuring 0.90–0.99 (0.95) x 1.99 – 2.37 (2.16) and have dorsally uninterrupted crown of 44 spines.

Oral sucker terminal, muscular, rounded in shape measuring 0.31 - 0.33 (0.32) x 0.19-0.22 (0.20). The pre-pharynx is absent. Pharynx elongated and muscular with an oval shape measuring 0.55–0.65 (0.61) x 0.45–0.54 (0.50).

Esophagus is moderately long, bifurcates above the ventral sucker into two intestinal caeca, reaching the posterior extremity of the body and measuring 0.61–0.69 (0.65) x 0.22–0.28 (0.25).

The acetabulum is muscular, rounded and larger than the oral sucker, located in the anterior part of the body measuring 0.71–0.74 (0.72) x 1.42–1.44 (1.43).

Ovary median, pre-testicular, rounded measuring 0.63–0.70 (0.67) x 0.91–0.92 (0.91). Seminal receptacle slightly larger than ovary, post ovarian measuring 0.71–0.73 (0.72) x 0.92–0.99 (0.95).

Testes post ovarian, tandem, unequal, intercaecal, multilobed, situated in the anterior part of hind body. Anterior testis measuring 1.32 –1.42 (1.36) x 0.94–0.98 (0.95) and posterior testis measuring 1.48–1.49 (1.48) x 0.77–0.78 (0.77). Cirrus pouch rounded lie between intestinal bifurcation and acetabulum measures 0.29–0.30 (0.29) x 0.77–0.97 (0.88). Genital pore median and post acetabular.

Vitellaria begins from posterior level of acetabulum, arranged in lateral fields and continued up to posterior level of excretory vesicle. The excretory pore sub terminal and excretory vesicle is J shaped.

Uterus intercaecal, with few coils starts from pre-ovary region and extend up to post acetabulum region.

Eggs oval, elongated, thin-walled, numerous measure 73.3–80.2 (76.96) x 98.3–113.2 (106.38).

DISCUSSION

Species of genus *Echinostoma* (Rudolphi, 1809) parasitize amphibian, birds, mammals and humans (Yamaguti, 1971). Hundreds of species have been reported to infest birds only in all continents of the world (S.Bushra *et al.*, 2014).

Twelve species of *Echinostoma* reported from Pakistan in different bird hosts are: *E. lahorensis* (Bhutta and Khan, 1974); *E. revolutum* (Froelich, 1802; Rudolphi, 1809; Bhutta and Khan, 1975); *E. chloropodis* (Bhutta and Khan, 1975); *E. megaovata* (Dharejo, 2006); *E. atrae* (Birmani *et al.*, 2008,); *E. sindhenses* (Dharejo *et al.*, 2009); *E. valentini* (Das and Ghazi, 2011); *E. unarii* (Saleem unar, 2011, unpublished part of thesis); *E. Rafiae* (S.Bushra *et al.*, 2014); *E. garzetti* (Bushra *et al.*, 2016); *E. cribbi* (Khan *et al.*, 2017) and *E. paraulum* (Memon *et al.*, 2018) and only a single species in mammals *E. bengalensis* (Shafi *et al.*, 1886).

New specimens is different from other species of genus *Echinostoma* (Rudolphi, 1809), in having 44 number of collar spines , while *E. kashmirensis* (Chisti and Ahmad 1991) has 25 spines ; *E. stridulae*, Reich, 1801; Dietz, 1909 has 27 spines; *E. ignavum* (Nicoll, 1914) has 29 spines; *E. anseris*, (Yamaguti, 1939) has 31 spines; *E. australe*, (Johnston, 1928) has 32 spines; *E. operosum* (Dietz, 1910) has 33 spines; *E. chasma* (Lal, 1939) has 34 spines; *E. aliud*, (Nicoll, 1914); *E. megaovata* (Dharejo, 2006); *E. valentini* (Das and Ghazi, 2011); *E. bhattacharyai* (Verma, 1936) have 35 spines; *E. koisarensis*, (Ablasov et Iksanov, 1960) has 36 spines; *E. revolutum* Rudolphi, 1809; Bhutta and Khan, 1975 has 37 spines ; *E. audyi* (Lie et Umathevy, 1965); *E. bancrofti* (Johnston, 1928); *E. caproni* (Richard, 1964); *E. columbae* (Zunker, 1925); *E. revolutum* (Froelich, 1802) Dietz, 1909 have 37 spines; *E. atrae* (Birmani *et al.*, 2008); *E. americanum* (Perez Viguera, 1944); *E. paraulum* have 38 spines; *E. cribbi* has 38-40 spines ; *E. aphyllactum*, (Dietz, 1909) has 39 spines; *E. rafiae*, *E. annulatum* (Cobbold, 1860) have 41 spines; *E. gracile*, (Perez Viguera, 1944) has 42 spines; *E. academicum* (Skrjabin, 1915) has 43 spines; *E. azerbaijanicum* (Kasimov, Vaidova et Feizullaev, 1959); *E. lahorensis*; *E. attenuatum* (Lumsden et Zischke, 1963); *E. australasianum* (Nicoll, 1914); *E. australasianum coromandum* (Odening, 1962) have 45 spines ; *E. chloropodis* (Bhutta and Khan, 1975); *E. chloropodis philippinense* (Tubangu, 1932) have 46 spines; *E. garzetti*, *E. chloropodis*, (Zeder, 1800) Dietz, 1909 have 47 spines; *E. unarii* (Saleem unar, 2011, unpublished part of thesis 2011) has 48 spines; *E. coecale* (Bashkirova, 1941) has 49 spines; *E. sindhensis* (Dharejo *et al.*, 2009); *E. armatum* (Molin, 1858) have 50 spines and *E. transfretanum* (Dietz, 1909) has 51 spines.

Body size in present specimens is (16.67–18.33 x 3.28-3.76) larger than below mentioned species; *E. parcespinosum* Lutz, 1924 (0.9–1.0); *E. turdi* Rudolphi, 1819, synonymized as *E. nephrocephalum* Cobbold, 1860 (1.05 x 1.06); *E. koisarensis* Ablasov et Iksanov, 1959 (1.07–1.3 x 0.25–0.33); *E. fragosum* Dietz, 1909 (2.1–3.12 x 0.3–0.33); *E. neglectum* Lutz, 1924 (2.5–4.5 x 1.0); *E. nephrocystis* Lutz, 1924 (2.5–4.5 x 1.0); *E.*

chasma (2.58 by 0.64); *E. columbae*, (2.63–7.65 x 0.85–1.53); *E. echinocephalum* (Rudolph, 1819) Cobbold, 1860 (2.7–3.3 x 0.23–0.27), *E. ignavum* (2.8 by 0.6), *E. aliud* (2.9–6.3); *E. dilatatum* Cobbold, 1840 (2–4 x 1.0); *E. crotophagae* de Faria, 1909 (3.0–8.0 x 1.0–1.9); *E. bhattacharya iindicum* Ablassov et Chibichenko, 1960 (3.12); *E. paraulum* (3.20–3.58 x 0.62–0.69) *E. americanum* (3.4 by 1.1); *E. asiaticum* Mendheim, 1943 (3.5–4.2 x 0.4–0.6); *E. hsui* Yamaguti, 1958 (3.5–4.2 x 0.4–0.6); *E. barbosa* Lee et Basch, 1966 (3.64–7.82 x 0.45–0.95); *E. oxycephalum* Rudolph, 1819 (3–4 x 1/3–1); *E. revolutum* (4.0–2.20 x 0.88–2.50); *E. azerbaijanicum* (4.5–4.8 x 0.8–1.2); *E. aphyllactum* (4.5–5.0 x 0.77–0.7784); *E. exechinatum* Solov'ev, 1912 (4.5–6.0 x 4.43); *E. emollitum* Nicoll 1914 (4.5–6.7 x 0.55–0.7); *E. goldi* Oshmarin, 1956 (4.0–7.0 x 1.36); *E. govindum* Moghe, 1932 (4.6–4.9 x 0.92); *E. mesotestius* Solov'ev, 1912 (4.75–8.32 x 0.99); *E. microrchis* Lutz, 1924 (5.0 x 1.0); *E. revolutum tenuicolle* Bashkirova, 1941 (5.12–8.0 x 1.8–2.0); *E. siticulosum* Dietz, 1909 (5.5–7.5 x 0.7–0.94); *E. exile* Lutz, 1924 (5.5–7.5 x 0.82–1.0); *E. caproni* (5.65 by 1.5); *E. bhattacharyai* (5.7 x 1.05); *E. ralli* Yamaguti, 1934 (4.5–8.8 x 0.8–1.3); *E. stridulae* (5–10 x 0.92–1.25); *E. longicirrus* Verma, 1936 (5–7 x 0.9–1.2); *E. crecci* Verma, 1936 (5–8 x 1.0–1.25); *E. minimum* Verma, 1936 (5 x 0.9); *E. elongatum* Nicoll, 1914 (6.0–9.5 x 0.7–0.8); *E. pekinense* Ku, 1937 (6.29–6.73 x 1.04–1.23); *E. chloropodis* (6.4–6.68 x 1.0–1.2); *E. sudanense* Odhner, 1910 (6.5 x 1.4); *E. nudicaudatum* Nasir, 1960 (6.83–7.63 x 0.94–1.28); *E. uitalicum* Gagarin, 1954 (6.8–7.5 x 1.35–1.75); *E. audyi* (6–14.1 x 0.81–0.5); *E. mendax* Dietz, 1909 (6–9.5 x 0.4–0.53); *E. condignum* Dietz, 1909 (7.0–13.5 x 0.95–1.3); *E. erraticum* Lutz, 1924 (7.0–8.8 x 1.45); *E. attenuatum* (7.22–11.22 x 0.88–1.59); *E. rousseti* Dollfus, 1956 (7.3 x 1.5); *E. chloropodis cachinnans* Dubois, 1951 (7.35–9.0 x 0.81–0.87); *E. chloropodis philippinense* (7.55–10 x 1.05–1.22); *E. stantschinskii caudatum* Yamaguti, 1939 (7.6–7.9 x 0.9); *E. cribbi* (7.60–8.22 x 2.05–2.22); *E. uralense* Skrjabin, 1915 (8.5 x 1.0); *E. academicum*, Skrjabin, 1915 (8.5 x 1.2); *E. necopinum* Dietz, 1909 (8.5–15 x 1.5–1.72); *E. robustum* Yamaguti, 1935 (8.8–9.8 by 1.33–2.18); *E. stantschinskii* Semenov, 1927 (9.0–9.8 x 1.14–1.29); *E. multispinosum* Perez Viguera, 1944 (9.4 x 1.3); *E. travassosi* Skrjabin, 1927 (9.48–9.68 x 2.12); *E. turkestanicum* Kurova, 1927 (9.65–10.9 x 1.8–2.33); *E. transfretanum* (9–11); *E. acuticauda* Nicoll, 1914 (9–12 x 0.9–1.0); *E. australe* (10.2 by 1.5); *E. uncatum*, Dietz, 1909 (10.5 x 1.98); *E. operosum* (10 x 1.4); *E. grande* Bachkirova, 1946 (11.25 x 1.5); *E. corvi* Yamaguti, 1935 (11.4 x 1.62); *E. alepidotum* Dietz, 1909 (11.5 x 1.14); *E. coecale* (11.7 x 2.07); *E. annulatum* (12 x 1.0); *E. gracile* (12.0 x 1.2); *E. amurzetium* Petrochenko et Egorova, 1961 (12.12–15.12 x 0.94–1.1); *E. kashmirensis* (13.04 x 1.55); *E. sindhenses* (13.7 x 3.7); *E. australasianum coromandum* (13.5 x 2.5); *E. anseris* Yamaguti, 1939 (13.20 x 2.1–2.5); *E. australasianum* (13–14 x 1.75); *E. armatum* (14 x 0.8); *E. atrae* (14.21–15.90 x 1.50–1.64); *E. hilliferum* Nicoll, 1914 (14.5–16 x 1.7–2.2); *E. uncinatum* (Rudolph, 1803) Cobbold, 1860 (14.6 x 2.25) and *E. sarcinum* Dietz, 1909 (14–14.5 x 2.08–2.14). *E. paracoalium* Sovetnikov, 1966 (17.1 x 2.3) and *E. bancrofti* (15.7 x 1.7) and *E. cribbi* (7.60–8.22 x 2.05–2.22)

Body Size of new specimens is smaller than *E. dietzi* Skrjabin, 1924 (19.107–21.23 x 1.69–1.89); *E. rufinae* Kurova, 1927 (19.82 x 1.69); *E. stromi* Bashkirova, 1946 (20 x 1.17); *E. miyagawai* Ishii, 1932 (21–26 x 2.0–3.5); *E. equinatum gigas* Marco del Pont, 1926 (35–40 by 3–4); *E. coronale* Kurova, 1927 (71.1 x 2.28); and *E. echinatum* Zeder, 1803 (92–134 x 59–76).

The eggs of present specimens are larger in size 98.3–113.2 x 73.3–80.2 than *E. rafiae* 0.05–0.07 x 0.03–0.04; *E. valentine* 0.062–0.04 x 0.072 x 0.04; *E. cribbi* 0.095–0.097 x 0.046–0.051; *E. garzetti* 0.09–0.012 x 0.05 x 0.056; *E. Revolutum* 0.63–0.089; *E. chloropodis* 0.104–0.107 x 0.071–0.76; *E. atrae* 10–15 x 11–86; *E. govindum* 47 by 23; *E. fragosum* 62.4 by 38.4; *E. hsui* 75 x 45; *E. asiaticum* 75 x 45; *E. americanum* 80 x 54; *E. stantschinskii* 80 by 65; *E. uncatum* 86.4–91.2 x 50.4–52.8; *E. aphyllactum* 84–87 x 48–50; *E. armatum* 90 x 60; *E. mesotestius* 93 x 50; *E. operosum* 88–93 x 60; *E. grande* 90–95 x 50; *E. multispinosum* 86–98 x 46–53; *E. chloropodis* 99 x 66; *E. australe* 80–100 x 60–70; *E. ignavum* 95–100 x 50; *E. mendax* 96–100 x 57–62; *E. condignum* 98.4–100.8 x 48–50.4; *E. uitalicum* 100 x 60–65; *E. crotophagae* 102 x 50; *E. australasianum coromandum* 81–103 x 44–59; *E. siticulosum* 93.6–103.2 x 50.4–55; *E. chloropodis philippinense* 101–104 x 75; *E. sindhenses* 86.5–105 x 62.5–70; *E. elongatum* 91–105 x 54–73; *E. stantschinskii caudatum* 96–105 x 63–72; *E. emollitum* 100–105 x 52–56; *E. unari* 85.5–105.0 x 57.5–70.0 (unpublished part of thesis); *revolutum* 90–105 x 59–83; *E. sudanense* 95–105 x 55–60; *E. azerbaijanicum* 83–108 x 48–72; *E. necopinum* 103–108 x 52–57; *E. anseris* 90–108 x 63–75; *E. chloropodis cachinnans* 95–108 x 65–72; *E. turkestanicum* 95–108 x 54–58; *E. pekinense* 100–108 x 54–58; *E. rufinae* 108 x 58; *E. coecale* 109 x 63–69; *E. bhattacharyai* 91–109 x 67–72; *E. exechinatum* 74–110 x 32–69; *E. bancrofti* 80–110 x 50–60; *E. amurzetium* 90–110 x 60–70; *E. alepidotum* 102–110.8 x 60–61.2; *E. goldi* 112 x 55 and *E. gracile* 112 x 80.

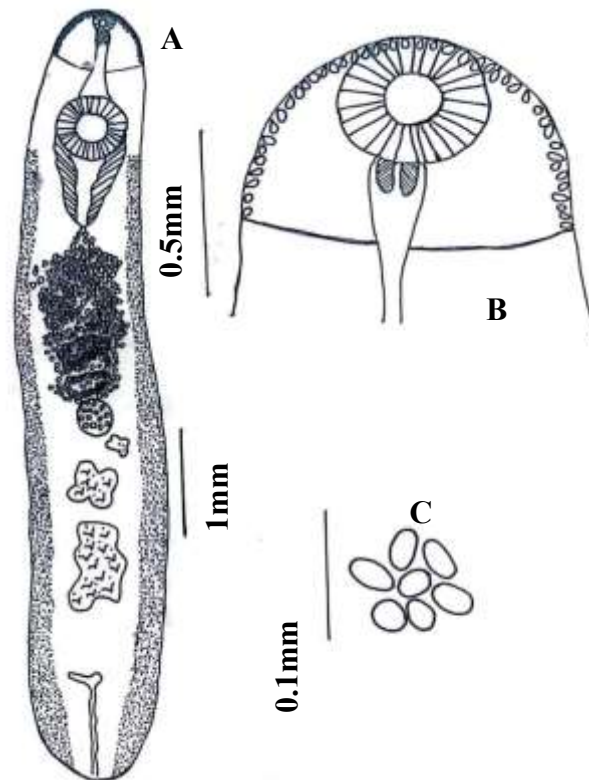


Fig. 1. *Echinostoma pakistanensis* sp. n.

- A. *Echinostoma pakistanensis* sp. n. Entire worm
 B. Head collar enlarged with oral sucker, pharynx and part of esophagus
 C. Eggs Enlarged.

Table 1. Comparison in avian hosts of genus *Echinostoma* (Rudolphi, 1809) described from Pakistan.

Species name	Host	Location	Body size (mm)	Egg size (mm)	No. of Spines
<i>E. lahorensis</i>	Day old chick	Lahore	Not mention	Not mention	45
<i>E. Revolutum</i>	<i>Anas platyrhynchos</i>	Balloki head work	7.05 x 1.121	0.63-0.089	37
<i>E. chloropodis</i>	<i>Gallinua chloropus</i>	Balloki head work	6 .66-10.9 x 0.818-1.332	0.104-0.107 x 0.071-0.76	46
<i>E. atrae</i>	<i>Fulica atra</i>	Manchar lake	14.21-15.90 x 1.50-1.64	10-15 x 11-86	38
<i>E. megaovata</i>	<i>Fulica atra</i>	Hyderabad	5.8 x 0.95	100-115 50-70	35
<i>E. sindhenses</i>	<i>Bubulcus ibis</i>	Hyderabad	13.7x 3.7	89.99-10.06 x 2-2.1	50
<i>E. valentine</i>	<i>Phalacrocorax fuscicolis</i>	Thatta	5.5-6.4 x 0.7-0.94	0.062 by 0.04 0.072 x 0.04	35
<i>E. unari</i>	<i>Egretta garzetta</i>	Jamshoro	13.33-14.86 x 2.66-2.80	85.5-105.0 x 57.5-70.0	48
<i>E. rafiae</i>	<i>Egretta garzetta</i>	Hyderabad	7.81-10.2 x 2.2-2.5	0.05-0.07 x 0.03-0.04	41
<i>E.garzetti</i>	<i>Egretta garzetta</i>	Jamshoro	9.99-10.06 x 2-2.1	0.09-0.012 x 0.05 by 0.056	47
<i>E. cribbi</i>	<i>Bubulcus ibis</i>	Matitari	7.60-8.22 x 2.05-2.22	0.095-0.097 x0.046-0.051	38-40
<i>E.paraulum</i>	<i>Corvus splendens</i>	Khairpur	3.20-3.58 x 0.62-0.69	Not Mentioned	38
<i>E. pakistanenis</i>	<i>Phalacrocorax fuscicolis</i>	Larkana	16.67-18.33 x 3.28-3.76	73.3-80.2 x 98.3-113.2	44

While eggs of new specimens are smaller in size than *E. attenuatum* 90–115 x 50–67; *E. revolutum tenuicolle* 94–115 x 55–64; *E. nudicaudatum* 97–115 x 67–72; *E. megaovata* 100–115 x 50–70; *E. stridulae* 108.8–116 x 60–68; *E. crecci* 109.2–117.6 x 58–69; *E. academicum* 118 x 74; *E. kashmirensis* 75–119 x 54–64; *E. travassosi* 107–119 x 51–64; *E. caproni* 105–120 x 50–60; *E. dietzi* 122–64; *E. aliud* 114–122 x 69–74; *E. sarcinum* 115.6–122.4 x 68–75; *E. transfretanum* 115.6–122.6 x 68–74.8; *E. paracoalitum* 114–123 x 44–57; *E. kashmirensis* 119–125 x 54–64; *E. stromi* 120–125 x 50–55; *E. erraticum* 125–67.5; *E. coronale* 125 x 57; *E. australasianum* 105–126 x 65–77; *E. acuticauda* 112–126 by 63–75; *E. corvi* 114–126 by 66–75; *E. minimum* 117–126 x 62–67; *E. uralense* 128 x 72; *E. robustum* 111–129 x 60–69; *E. columbae* 130 x 70; *E. ralli* 110–130 x 68–81; *E. hilliferum* 120–130 x 70–75 and *E. audyi* 98–132 x 60–75.

CONCLUSION

The present specimens differ from all recognized species of the genus in distinct morphometric features. These morphotaxonomic features show variation in body, spines, oral and ventral sucker, eggs size, shape and position. Consequently, it is proposed to be new as *Echinostoma pakistanensis* sp. n. The name of the species in honor of the host country

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