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Research Article

Description of an Indian Population of *Urosomoida perthensis* (Ciliophora: Oxytrichidae) with 16 FVT Cirri from a Pond located at Rajghat, Delhi, India

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Abstract

Ciliates constitute a vast monophyletic group of unicellular eukaryotes. However, the knowledge and understanding of ciliate taxonomy and biodiversity are still limited, especially from the Indian subcontinent. The present study aims to report an Indian population of *Urosomoida perthensis* [16], which is a new record to the Indian fauna. *U. perthensis* was isolated from a man-made pond located in the Rajghat, Delhi India. The species of the genus *Urosomoida* are indistinguishable from the species of genus *Oxytricha* in live cell as both have flexible bodies. However, it differs from *Oxytricha* in two features i.e. lack of fragmentation of 3rd dorsal kinety (DK_3) and the presence of less than 18 frontal-ventral-transverse (FVT). These two features are the autapomorphies of this genus. This genus is placed in "non-oxytrichid Dorsomarginalia" because they lack fragmentation of dorsal primordium (DP_3). The morphology and ontogenesis of Indian population of *U. perthensis* is described on the basis of protargol impregnation. Some of the morphological salient features of *U. perthensis* are as follows: size of protargol stained cells- ~50 x 14 µm; anterior rounded end and posterior tapering end; 2 macro-nuclear nodules and 2 micronuclei; 16 Frontal-Ventral-Transverse (F_{1-8}, V_{1-5}, T_{1-3}) cirri; four dorsal rows of bristles- three dorsal kineties (DK_{1-3}) and one dorso-marginal (DM_1); No split in DP_3; 3 caudal cirri, a gap in DK_1 continuity; on an average 17 adoral membranelles; 16 right marginal cirri; 13 left marginal cirri; flexible body; buccal cavity flat and narrow and undulating membranes (UM_S) in the typical *Oxytricha* pattern; morphogenesis in typical *Urosomoida* pattern [11]; Primordia V & VI of proter are formed by the anterior movement of kinetosomes of primordial V & VI of opisthe.

Keywords: Oxytrichidae, *Urosomoida*, Morphology, Morphogenesis

Introduction

Ciliates (phylum: Ciliophora [10]) constitute a vast monophyletic group of unicellular eukaryotes that are studied across multiple biological fields [8, 19, 25, 29, 34, 36, 39]. However, there is little knowledge and comprehension of ciliate biodiversity and taxonomy, and it is estimated that 83–89% of free-living ciliate species are still unknown.-[15, 17]. The ciliate species have also not been thoroughly investigated yet in the Indian subcontinent. In the present study an Indian population of *Urosomoida perthensis* [16] was isolated for the first time. Genus *Urosomoida* was created by Hemberger in Foissner (1982) with *U. agilis* (Engelmann, 1862) as type species. Previously, *Urosomoida* was classified under the family Oxytrichidae, whose type genus *Oxytricha* has 18 frontal-ventral-transverse (FVT) cirri with dorsal kinety 3 fragmentation. However, *Urosomoida* has reduced FVT cirri than the typical Oxytricha and lacks the fragmentation of dorsal kinety 3. Therefore, this genus was placed in the non-monophyletic assemblage "non-oxytrichid Dorsomarginalia" [3, 4].

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The species of this genus are indistinguishable from the members of *Oxytricha* in live cells. They are similar to the members of genus *Oxytricha* in many other morphological features too. However, the genus shows 2 autapomorphies; the distinct reduction of the number of pre-transverse ventral cirri and transverse cirri and absence of fragmentation of 3rd dorsal kinety [2]. Earlier the ontogenetic feature "primordia of V and VI of proter originate *de novo*" was also included in the characterization of the genus *Urosomoida* [2, 5]. However, there is scarcity of ontogenetic data in *Urosomoida*, therefore this characteristic was eliminated from the diagnosis until further analysis and it requires more ontogenetic data[33]. So, an improved diagnosis of the genus *Urosomoida* was provided by Shao and colleagues [33].

The genus *Urosomoida* comprises of 16 species. These species are *U. agilis* (Engelmann, 1862; Hemberger in Foissner, 1982), *U. agiliformis* [11], *U. minima* [24], *U. perthensis* [16], *U. antarctica* [12], *U. granulifera* [12], *U. deserticola* [14], *U. namibiensis* [14], *U. monostyla* [14], *U. reticulata* [14], *U. pseudofurcata* [2, 14, 33], *U. halophila* [13], *U. galapagensis* (Foissner and Liebtreu in Foissner, 2016), *U. marcili* [9, 33], *U. paragiliformis* [37] and *U. sejongensis* [26]. Most of the species of this genus are usually reported from fresh water ecosystems but some species have been found in lagoons or extremely saline soil [2, 9, 14].

The objective of the current study is to describe the morphology and morphogenesis of an Indian population of *Urosomoida perthensis*, that was found in a man-made pond located at Rajghat, Delhi, India. For the first time, this species has been reported from the Indian subcontinent and added a new record to the fauna of the county.

2. Materials and Methods

a) Water sampling and their processing: Water samples were periodically collected from a man-made pond, located in Rajghat, Delhi, India. These were brought to the laboratories for further processing. Nytex nets of 60-120 µm were used to filter out large crustaceans. Mixed cultures were grown at room temperature and fed with organism *Chlorogonium* and periodically examined for ciliates.

b) Culturing and maintenance of Cells: To create clonal cultures of the species under study, live ciliates were observed and isolated under the microscope. They were cultured in Pringsheim's medium [7, 32]. The composition of the Pringsheim's medium was $Ca(NO_3)2.4H2O$ (0.85mM), KCl (0.35 mM), $MgSO_4.7H2O$ (0.08mM) & $Na_2HPO_4.2H_2O$ (0.11mM). *Chlorogonium elongatum*, a green alga, was given to the cells every day as food. The temperature of the cells was maintained at 23 ± 1 ºC in B.O.D. with enough feeding.

c) Staining of the cells: The method employed was protargol impregnation to examine the organism's infraciliature, adhering to the previously indicated methods of Wilbert [38] as well as slightly modified methods of Kamra and Sapra [27].

Regular Bouin's fixative was used to fix the ciliates. After being fixed, the cells were coated with albumin-glycerine mixture and bleached in 0.6% sodium hypochlorite (NaOCl) solution. Following that, the cells were then stained with 2% freshly prepared Protargol. To enable the stain to develop the desired colour after impregnation, the slides were dipped in the "slow developer" solution, which was a deviation from the original protocol. After that, the slides were immersed in 5% sodium thiosulphate ($Na_2S_2O_3.5H_2O$) to fix the silver stain. Finally, the slides were cleaned with xylene, dehydrated with alcohol, and mounted in D.P.X.

d) Morphometry and morphogenesis: Though the species *Urosomoida perthensis* was discovered and reported earlier [16] along with its morphology and morphometry, its developmental morphogenetic characterization, description and reporting of the Indian population is being done for the first time in the current investigation.

Protargol impregnation was used to examine the organism's infraciliature, adhering to the previously indicated methods of Wilbert [38] and Kamra and Sapra [27]. Twenty impregnated cells were counted and measured using an oil immersion objective lens (1000X) after the silver

staining. The present study adhered to the standard reference studies' terminology for describing the species, such as Berger [4], Foissner and Stoeck [18], and Küppers et al. [28]; on the other hand, the cirri numbering system employed was the one created by Wallengren [35], Borror [6], Martin [30], and Hemberger [24].

3. Results: The species under investigation is the Indian population of *Urosomoida perthensis* reported for the first time from India.

3. (a). Occurrence: *U. perthensis* was found in a man-made pond located at Rajghat, Delhi, India.

3. (b). Diagnostic features: Size of protargol stained cell 50x14 µm; anterior rounded end and posterior tapering end; 2 macronuclear nodules and 2 micronuclei; 16 Frontal-Ventral-Transverse(F_{1-8}, V_{1-5}, T_{1-3}) cirri; four dorsal rows of bristles (DK_{1-3} and DM_1); No split in DP_3; 3 caudal cirri, a gap in DK_1 continuity; on an average 17 adoral membranelles; 16 right marginal cirri; 13 left marginal cirri; flexible body; buccal cavity flat and narrow and undulating membranes (UM_S) in the typical *Oxytricha* pattern; morphogenesis in typical *Urosomoida* pattern (Hemberger in Foissner, 1982); Primordia V & VI originate are formed by the anterior movement of kinetosomes of primordial V & VI of opisthe.

3. (c). Description of the species **(Figs. 1A & D)**: Average size of a protargol impregnated non-dividing cell is 50x14 µm. The anterior end is rounded while the posterior is tapering. Cells are very flexible, dorso-ventrally flattened with length to width ratio of 3.5:1. Each of the two macro-nuclear nodules measures 7x5 µm and are located on the left of the median line of the cell. Two spherical compact micronuclei measure 3.5 µm each and are attached to macro-nuclear nodules at variable positions. Cells show rapid movements. These are generally bottom dwellers. Conjugation and encystment is very frequent. The cysts are covered with blunt spines. The diameter of the protargol stained cyst is about 8.68 µm. Morphometric characters of Indian population of *Urosomoida perthensis*, are shown in table 1.

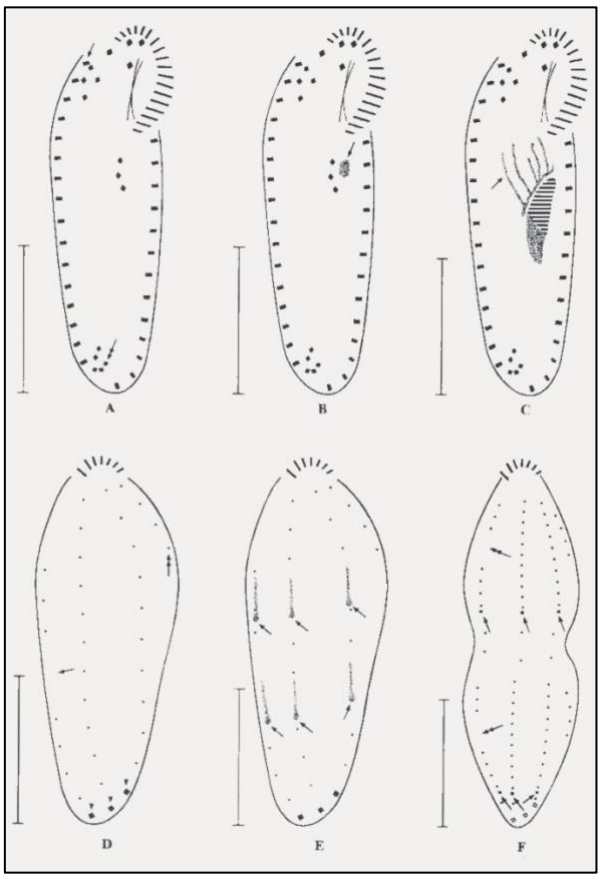

Figure 1: Line diagrams of protargol impregnated cells of Indian population of *Urosomoida perthensis* (Foissner & O'Donoghue, 1989) showing vegetative surface (A & D) and division morphogenesis on ventral (B & C) and dorsal (E & F) surfaces. **A.** Vegetative ventral surface: Beginning of RMC row (arrow), presence of three transverse cirri (double arrow). **B.** Origin of OP (arrow). **C.** Anterior movement of streaks V & VI of opisthe (arrow). **D.** Gap in DK_1 (arrow), only one DM (double arrow). **E.** Within row proliferation of 2 sets of 3 DP and localized proliferation of kinetosomes at their posterior ends (arrows). **F.** Formation of new caudal cirrus at the end of $DK_{1,2\&3}$ (arrows), presence of gap in DK_1 (double arrows). Bar represents 20 µm

The AZM is shaped like a question mark (**?**), and comprises approximately 17 membranelles. It occupies 30% of the body length. The buccal cavity is flat and narrow. The undulating membranes (UMs) are in the typical *Oxytricha* pattern. The ventral ciliature consists of 16 FVT cirri. Eight frontal cirri are arranged in two groups wherein anterior frontals F_{1-4} are slightly larger than the posterior ones. The posterior frontals F_{6-8} appear in a 'V' shaped pattern. The three postoral ventrals are almost equidistant lying near the cytostome, arranged in a row. The V_5 is present immediately above the level of T_1 while V_4 is

distant from it. There are three transverse cirri, which lie close to the right margin of the cell. Two of these T_2 and T_3 are very close to each other while T_1, lies slightly distant from them. The right marginal cirral row starts at the level of F_5 and terminates at the level of pre-transverse ventrals and sometimes appears continuous with the transverse cirri. The 'J' shaped left marginal row terminates near the center of the cell. The dorsal ciliature consists of 4 dorsal rows of bristles (DK_{1-3} and DM_1). The DK_{1-3} elongate over the length of the whole body. DK_1 and DK_2 start below the level of DK_3. The DK_1 consists of 5-9 bristles, which are present in two discontinuous stretches. Two to three bristles are present in the anterior stretch while the remaining bristles are present in the posterior stretch with a gap in the middle of the row. There is a single short dorsomarginal row consisting of 3-5 bristles. Three caudal cirri are present one each at the end of DK_{1-3}.

3. ***(d). Divisional morphogenesis*** **(Figs. 1B-C &; Fig.2 A-D):** Kinetosomes appear *de novo* and leads to the formation of oral primordium (OP) in the region between the left marginal row and the post oral ventral cirri. The site of origin is very close to the first postoral ventral cirrus (V_1). Initially the origin is de-novo and soon, the kinetosomes of V_1 are also incorporated. Two sets of six FVT ciliary streaks are formed from OP and six parental cirri, which include three frontals ($F_{1, 7 \& 8}$) and three ventral (V_{1-3}) cirri. Initially V_2 forms one primordial streak and later, kinetosomes from OP are incorporated and four streaks are formed. Then V_3 disaggregates and its kinetosomes are also incorporated into the OP and finally all six streaks are formed. Movement of the kinetosomes from OP has been observed up to the UMs but their involvement in the formation of streak II of proter has not been confirmed. In the proter disintegrating UMs function as streak I. F_1 disintegrates into streak II. Streak III originates from the disintegration of F_8 while streak IV originates from F_7. The streaks V and VI of opisthe migrate anteriorly to form the streaks V and VI of proter. The 16 FVT cirri differentiate in the following the pattern of 1,2,2,3,4,4. The second and third streaks form only two cirri and do not contribute to the formation of transverse cirri.

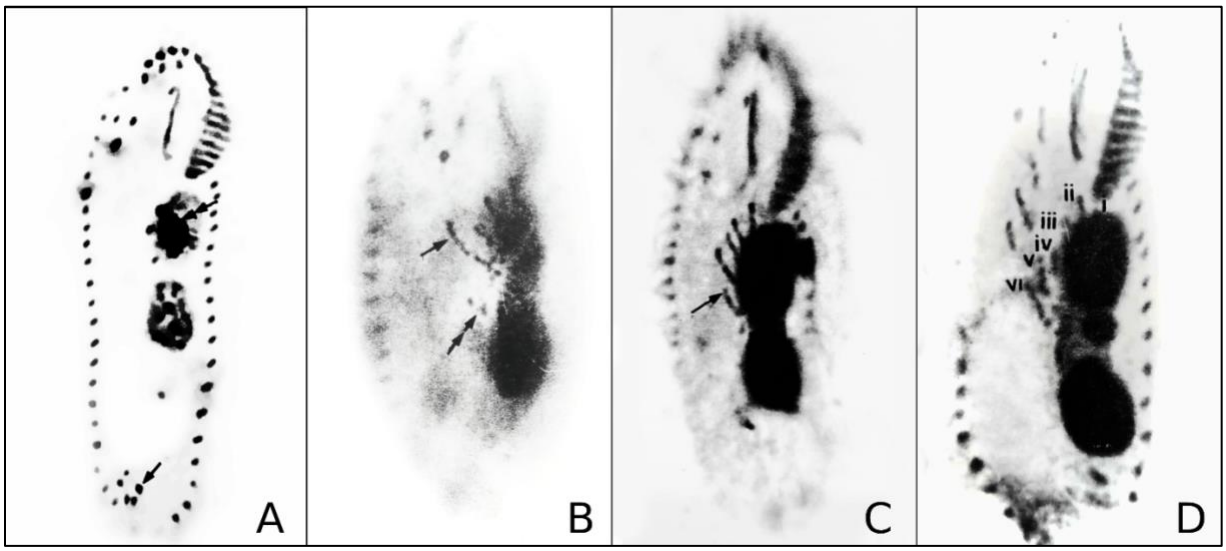

Fig. 2: Photomicrographs of protargol impregnated cells of Indian population of *Urosomoida perthensis*. **A.** Presence of only 3 transverse cirri (arrow), Origin of OP between LMC & POVS (double arrow); **B.** Streaks (arrow) formed from opisthe, disaggregation of V_3 (double arrow); **C.** Six streaks originated from OP and disaggregation of V1-3 (arrow); D. Full set of six streaks (i-vi)

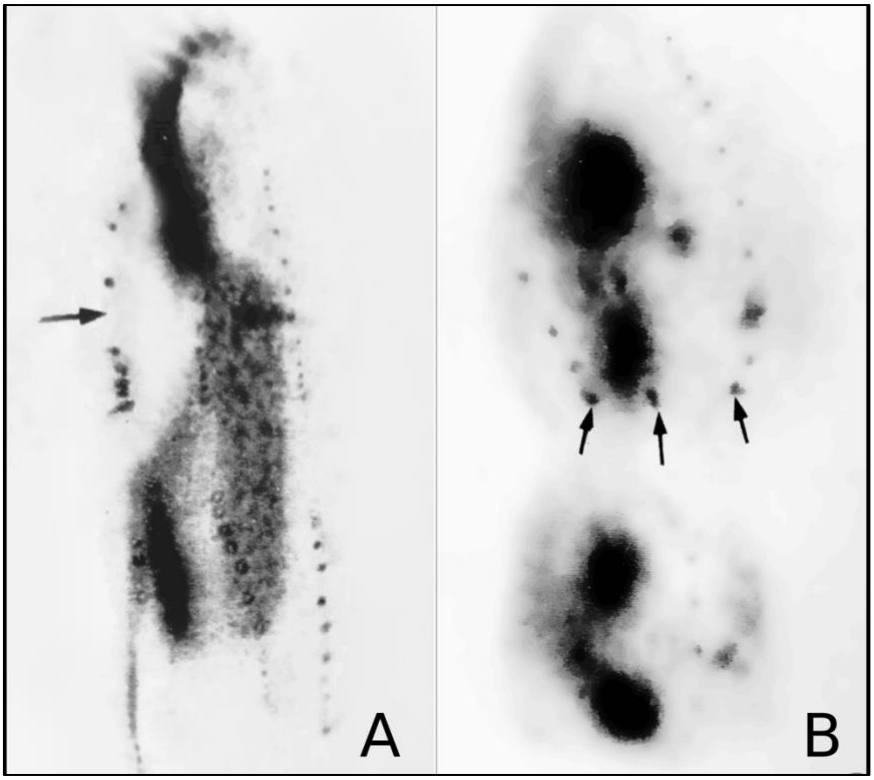

Fig.3: Photographs of protargol impregnated cells of Indian population of *Urosomoida* perthensis. A. Presence of a gap in the continuity of DK_1 (arrow); B. Formation of one caudal cirrus each at the end of $DK_{1,2\&3}$ (arrows)

Within-row kinetosome proliferation forms the marginal rows as described earlier for the sub-family Oxytrichinae [1, 2, 20, 21, 22, 23, 31]. On the dorsal surface (Fig.1 E-F; Fig. 3A-B), initially two sets of three primordia are formed by within row proliferation of kinetosomes. These primordia elongate, differentiate and develop into three new rows of dorsal kineties (DK_{1-3}). There is no split in third dorsal primordium so only three dorsal kineties are formed. The differentiated DK_1 bearing 6-7 bristles splits into an anterior part consists of 2-3 bristles while the remaining bristles are present in the posterior part. The gap in the DK_1 enlarges as the cell enters cytokinesis. At the posterior end of each dorsal kinety (DK_{1-3}), a caudal cirrus is formed. The short dorso-marginal is formed at the ventral surface close to the RMC primordia and then shifts to the dorsal surface.

Table 1: Morphometric analysis of the Indian population of *Urosomoida perthensis*

Characters	Mean	Min	Max	SD	CV	n
Body length	50.4	45.9	57.4	3.11	6.16	20
Body width	14.4	11.8	18.6	1.75	12.15	20
Body length/ Body width	3.5	2.7	4.3	0.53	15.01	20
Number of Macronuclei	2.0	2	2	0.00	0.00	20
Length of Macronucleus	7.5	6.3	9.9	0.87	11.66	20
Width of Macronucleus	5.4	4.3	6.5	0.48	8.82	20
Number of Micronuclei	2.0	2	2	0.00	0.00	20
Diameter of Micronucleus	3.5	3.1	3.7	0.17	4.91	20
Number of AM	16.6	14	19	1.19	7.15	20
Adoral length	14.8	12.7	18.4	1.37	9.23	20
Adoral length/ Body length	0.3	0.2	0.4	0.04	13.33	20
Number of Frontal cirri	8.0	8	8	0.00	0.00	20
Number of Ventral cirri	5.0	5	5	0.00	0.00	20
Number of Transverse cirri	3.0	3	3	0.00	0.00	20
Number of LMC	13.4	11	17	1.6	11.97	20
Number of RMC	15.8	13	21	2.28	14.46	20
Number of DKs	3.0	3	3	0.00	0.00	10
Number of DMs	1.0	1	1	0.00	0.00	10
Number of Dorsal bristles	DK_1	6.8	5	9	1.06	15.59	10
	DK_2	11.3	8	13	1.21	10.76	10
	DK_3	9.7	8	12	1.23	12.75	10
	DM_1	3.6	1	6	1.43	40.28	10
Number of Caudal cirri	3.0	3	3	0.00	0.00	10

4. Discussion

4(a). Distribution and ecology: The Indian population of *Urosomoida perthensis* was obtained from a man-made pond located in Rajghat, Delhi, India.

4(b). Comparison of Indian population of Urosomoida perthensis with Urosomoida perthensis (Table 2)**:**

Table 2: Comparative analysis of Indian population of *Urosomoida perthensis* & *Urosomoida perthensis*

Character	**Indian population of *Urosomoida perthensis***	***Urosomoida perthensis*** [16]
Body shape	Anterior end rounded while the posterior is tapering	Long elliptical
Body length	50.4 µm in protargol impregnated cells	50-70 µm in life
Body width	14.4 µm	20-30 µm in life
Length of Macronucleus	7.5 µm	7.3
Width of Macronucleus	5.4 µm	5.7
Number of Micronuclei	2	1 (From Diagram)
Diameter of Micronuclei	3.5 µm	2.7x2.4
Number of AM	16.6	16.6
Number of LMC	13.4	16.4
Number of RMC	15.8	19.5
Number of Dorsal Kineties	4	4
OP origin	De novo; between LMC & POVC	Not mentioned in literature
Caudal cirri	3	3
Cyst surface	Spiny	Not mentioned
Sub-pellicular granules	Absent	Absent
Occurrence	Man made pond	Pond & soil

Both, the Indian population of *Urosomoida perthensis* as well as *Urosomoida perthensis*, consist of 16 FVT cirri.

4.(c) (i) Morphology & Morphometry: Both the species are very similar to each other in their morphometric features such as their body length, body width, macro-nuclear number and their size, number of caudal cirri and the absence of sub-pellicular granules, shown in the above table (Table 1).

On the dorsal surface, both the species consists of 3 dorsal kineties and one dorso-marginal row, which is a peculiar feature of the genus *Urosomoida.*

The most important resemblance in Indian population of *Urosomoida perthensis* as well as *Urosomoida perthensis* [16] is that, both the species consists of four dorsal rows of kineties and DK_1 shows a conspicuous gap in the middle which is due to the lack of 2-3 basal bodies. The upper part consists of only 2-3 bristles while the remaining bristles are present in the lower part. The bristle free gap in DK_1 results due to the resorption of either one or sometimes two bristles after breakage of the kinety. Before it breaks 8-9 bristles are present in a continuous row but afterwards when a gap develops only 6-7 bristles are present. Thus, in this process 2-3 bristles are resorbed.

4.(c) (ii). Morphogenesis: Morphogenesis of Indian population of *Urosomoida perthensis* as well as *Urosomoida perthensis* [16] is in typical *Urosomoida* pattern. That means both the species do not show the fragmentation of third dorsal primordia and therefore only 3 dorsal kineties are present on dorsal surface.

In case of genus *Urosomoida*, on the ventral surface, the origin of streaks V and VI of proter is de-novo as reported in the literature. However, in the present study kinetosomes of streaks V and VI of opisthe migrate anteriorly and form streaks V and VI of proter and this movement of the kinetosomes of streaks V and VI has been confirmed in all the cells.

16 FVT cirri differentiate following the pattern 1,2,2,3,4,4. The second and third streaks form only two cirri and do not contribute to the formation of transverse cirri.

5. Conclusion: Due to these similarities the species under investigation is considered as *Urosomoida*

perthensis, which is first time reported from the Indian sub-continent.

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Statements and Declarations

All authors of this article have made substantial contributions towards the conception and design of the work, acquisition, analysis, and interpretation of data; as well as for the preparation of draft and editing of the manuscript and approved the final version for submission.

Competing Interests: The authors further declare that-

i. there are no financial or non-financial interests that are directly or indirectly related to the work submitted for publication,

ii. the work was not supported by any governmental or non-governmental financial institutions, and

iii. the authors declare no conflicting interests.

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