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Percentage prevalence and morphological details of *Stylonychia pustulata* (Ehrenberg, 1830) in the fresh waters of Nashik district

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ABSTRACT

The ciliates are one of the most important groups of Protozoa, common everywhere in waters of lake, pond, oceans and soils with many ecto and endosymbiotic members, as well as some obligate opportunistic parasites. Free living ciliates are found in fresh, marine, estuarine waters and in the soil. The present species described as *Stylonychia pustulata* Ehrenberg (1830), (O. F. Muller, 1786) Ehrenberg, 1835 (O. F. Muller, 1786) Ehrenberg, 1838, *S. pustulata* (Deshmukh, 2010) has found in fresh water ponds, ditches and streams, especially more abundantly in rain water bodies of Nashik district.

Introduction

The ciliates are one of the most important groups of Protozoa, common almost everywhere there is water (lake, pond, and oceans) and soils with many ecto and endosymbiotic members, as well as some obligate opportunistic parasites. Free living ciliates are found in fresh, marine, estuarine waters and in the soil.

An excellent source is a shallow semi permanent pond in a farm yard. Any stream large or small, or any freshwater hole, temporary or permanent, water at the base of aquatic plants is likely to be a valuable source of material. Such places as moist crotches or bark (crevices) in trees or any other moist or water containing silt, floating algal mats provide food. Cilia covering the body of the organism or a part of it, a major defining characteristic of this group and hence the phylum name "Ciliophora".

The ciliates possess a marked variety of motile systems based on ciliary motion. Thus, the organisms are able to move through the environment in search of prey often with remarkable agility and swiftness, or when sedentary, to create a flow of water carrying food to the organism. The arrangement of hair like organelles on the cell surface and the degree of coordinated action made possible by close proximity of the cilia varies considerably among the groups.

A cirrus is a composite group of cilia (a few to hundreds of cilia) often tapering toward the distal end and acting in unison. Typically they are found in localized regions on the cell surface. When the cirri are largely localized on the ventral and anterior surfaces, the ciliates are characterized as hypotrichs, signifying that the cirri are positioned beneath the cell. In other species, cilia are arranged in spirals (e.g. Spirotrichs) or in ribbon like assemblages near the anterior (sometimes broader end) of the cell. Although many ciliates exhibit rapid and impressive locomotion, others such as suctorian are sedentary and lack cilia during most of their life cycle. These feeding (trophont) stages are anchored to the substratum by a stalk or other form of holdfast.

Ciliates in general have great power of regeneration, and the process like reproduction is more complex, a missing part can be replaced by simple growth. Unlike eukaryotes, ciliates have two different nuclei; large polyploidious macronucleus and smaller diploid micronucleus. The former is important for protein synthesis, and latter for reproduction. The macro nuclei must be regenerated from the micronuclei. In most, this occurs during sexual reproduction which is not usually through syngamy but through conjugation. Joblet (1645-1723), has done pioneer work on ciliature, nuclei and contractile vacuoles in ciliates. In the eighteenth century Muller (1773, 1776 and 1786) gave the first extensive taxonomic account of all protozoa.

Extensive studies have been made during the last 80 years, with numerous outstanding and noteworthy contributions in the field of Protozoology, covering cytology, cytochemistry, structure, life cycle transmission, evolution, and ultrastructure; and inter relationship a verity of protozoa.

Although all ciliates conform, more or less, to the principle of nuclear dimorphism, they vary greatly in shape, size and number of nuclei. The macronucleus may be single compact, rounded or kidney shaped,

In reproduction by binary fission, the macro and micronuclei, either or both, divide; so that the parental nuclei are equally represented in both daughters. During sexual process of conjugation, two individual (the conjugant) unite temporarily. Micronucleus, or micronuclei, of both divide twice. All but one of the resulting micronuclei in each conjugant degenerate and disappear while the remaining one divides unequally forming two pronuclei. The smaller one is active, migratory, male pronucleus and the bigger one stationary, female, pronucleus. The male pronucleus migrates into the other conjugant and fuses with its stationary pronucleus forming a zygote nucleus or syncaryon. The two conjugants then, separate and are known as the exconjugant. These differ from ordinary individuals in having only a single nucleus, for the old macronucleus by now is in the process of losing its identity and being absorbed into the cytoplasm. The way in which the macronucleus disappears, varies considerably in different ciliates. During subsequent

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divisions the syncaryon gives rise to both macro and micronuclei.

Endoplasm is the seat of the main metabolic processes. The endoplasm is completely enclosed by cortex except at two places where it is brought into contact, temporarily or permanently, with the outside world. These are the cell mouth (cytostome) and cell anus (cytoproct). Food is taken in by the cytostome and surrounded by a vacuole into which digestive enzymes are secreted. The vacuole circulates slowly in the interior of animal during which time its cintents are digested and absorbed through its wall and finally brought to the site of cytoproct which opens to discharge the undigested matter. In some ciliates the part of the endoplasm immediately adjoining the mouth forms a fairly well defined oesophagus like tube and among some groups of holotrichs this is surrounded by a series of parallel rods (trichites) of unknown origin, sometimes elaborate enough to form a basket like structure.

One or more contractile vacuoles are present typically in freshwater ciliates to regulate ion balance of the cytoplasm and expel excess water. Various extrusomes (ejectile organelles) occur beneath the surface of the cell including mucocysts that secrete surface substances either to coat the cell to improve its immediate environment, provide material for cyst walls or aid in the capture of prey.

Most ciliates feed on smaller organisms, such as bacteria, algae and detritus swept into the mouth by modified oral cilia. Feeding consist of two processes each of which may limit the actual rate of feeding. The first one is the process of phagocytosis, i.e. the enclosure of a food particle in a membrane covered vacuole in which digestion takes place. In ciliates this occurs at a special site on the cell surface called cytostome or cell mouth. Cytostome is simply a two dimensional aperture and most commonly permanently open. It is covered by a single unit membrane from which food vacuoles are formed. Food materials first pass into the endoplasm of the organism via a more or less distinct cytopharynx. It may open directly to the exterior or be sunken into a depression or cavity or some kind, such as atrium, vestibulum or buccal or peristomial cavity.

The cytostome is often associated with various organelles in the surrounding cytoplasm in particular bundles of microtubules. Later play a role in the transport of captured particles on the surface surrounding the cytostome. A variety of ciliary's and other organelles serve to concentrate or retain food particles.

Material and Methods-

For the study, the water samples were collected from different parts of Nashik dist (Deola, Kalwan Nandgaon; Surgana, Satana) of Maharashtra state. Water samples were collected in wide mouth, sterilized glass bottles. Due care was taken and the samples were collected from where the submerged plants and decaying leaves were present. Mostly the samples were collected during morning and evening. The temperature of the sample bottles were maintained with the help of ice bags.

Rapid movements of ciliates make it difficult to identify ciliate species. To immobilize their movements methyl cellulose solution was used.

Culture methods –

For cultivation various media are used such as

- Hay infusion
- Wheat infusion
- Rice infusion

Hay infusion was good for *Stylonychia* and many other ciliates species. Rice grain infusion is also best for the *Vorticella, Stylonychia* and *Coleps* species.

Water sample is obtained from different water bodies contains a mixer of organism, by adding any type of culture media (Wheat or rice infusion) can generally rise the number of certain ciliate species (by rising the bacterial population present)in the water sample, but often lower the number of species. However the rough sample containing several ciliates and unknown bacterial and algal floras can be obtained for several weeks. Selection of a particular ciliate species and culturing it in the isolation of organisms other than its prey may obtain a greater degree of control over a culture.

The choice of culture medium depends largely upon what the ciliate feeds. Many feed upon bacteria and that is why many of the media commonly used are designed to encourage the growth of bacterial populations. Different culture media and methods published by Mackinnon and Hawes (1961), Kirby (1950) and committee on cultures, Society of Protozoologist (1958).

Culture were examined under low power and then in to high power microscope taking care to focus at all levels in the culture watching for movements of any kind. Movements of ciliates were also trapped in video camera for the proper understanding. Dry silver impregnation was used to study infraciliature of the ciliates. For the permanent slides the ciliates were fixed in terms of number of individuals and species composition. Methyl cellulose has been found too many advantages, as it arrest the movement, ciliates can be identified by their appearance. The species identification has been made mainly on the basis of arrangement of cilia, size and shape of body and structure of macro and micronucleus.

Discussion and Results-

Percentage of prevalence of *Stylonychia pustulata* in fresh water during the period of January 2007 to December 2008, for the two years

Table 1. Showing month wise prevalence of *Stylonychia* Sp. in freshwaters of Nashik District during the year 2007

Sr No	Month	Total No.of samples Examined	No.of .+ve samples	% of Total Prevalence
1	Jan-07	45	6	13.33
2	Feb-07	55	7	12.73
3	Mar-07	47	6	12.77
4	Apr-07	60	6	10.00
5	May-07	61	5	8.20
6	Jun-07	67	15	22.39
7	Jul-07	54	21	38.89
8	Aug-07	57	20	35.09
9	Sep-07	58	12	20.69
10	Oct-07	60	11	18.33
11	Nov-07	64	10	15.63
12	Dec-07	49	7	14.29
	Total	677	126	18.61

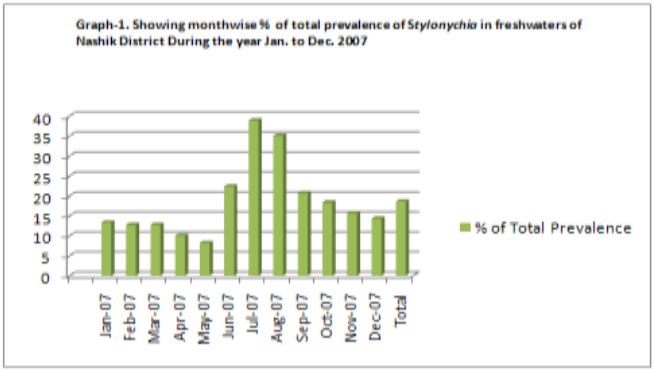
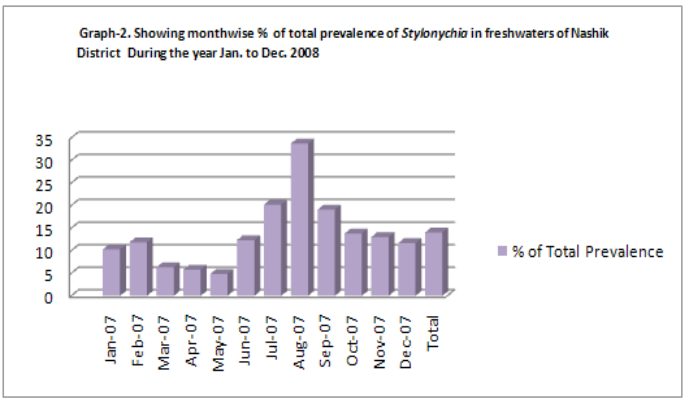


Table 2. Showing month wise prevalence of Stylonychia in freshwaters of Nashik District During the year 2008

Sr No	Month	Total No.of samples Examined	No.of .+ve Hosts	% of Total Prevalence
1	Jan-08	68	7	10.29
2	Feb-08	84	10	11.90
3	Mar-08	79	5	6.33
4	Apr-08	69	4	5.80
5	May-08	62	3	4.84
6	Jun-08	89	11	12.36
7	Jul-08	94	19	20.21
8	Aug-08	80	27	33.75
9	Sep-08	89	17	19.10
10	Oct-08	87	12	13.79
11	Nov-08	69	9	13.04
12	Dec-08	77	9	11.69
	Total	**947**	**133**	**14.04**



During the period of two years (Jan.2007 to Dec. 2008) a total number of water samples 1624 were examined, 259 of these were positive with free living ciliate *Stylonychia pustulata*.

In the first year (Jan.2007 to Dec.2007) 677 fresh water samples were examined, 126 of these were positive with free living ciliates. The percentage of prevalence of free living ciliates was about 18.61%.

In the second year (Jan.2008 to Dec.2008) 947 fresh water samples were examined, 133 of these were positive. The percentage of prevalence of free living ciliate *Stylonychia* was about 14.04%.

A month wise analysis in **first year** (Jan.2007 to Dec.2007) shows the maximum percentage of prevalence during August (33.75%) and minimum in May which was only 8.20%. and minimum to moderate in remaining months.

During the period of Jan. 2008 to Dec.2008) maximum percentage of prevalence of this ciliate was showed again during July (33.75%) and lowest in May (4.84%) and minimum to moderate in remaining months.

The pattern in both the years suggests that the peak is soon after the monsoon rain. The percentage then gradually reduces at the end of the winter months and reaches a low with the onset of summer. The details of the number of water samples examined and the month wise prevalence is shown in **Table No1 and 2.**

Description of the species

Synonym of *Stylonychia* was described by O. F. Muller (1786) later Kerona, the Andre, (1916) had given the name *Prosopsenu.* Ehrenberg, (1838) included this genus in Family Oxytrichidae. The species which are included in genus Stylonychia are inflexible, elongate, oval, dorso-ventrally flattened body with a large and powerful AZM supported anteriorly by a collar. 2 rows of marginal cirri not continuous posteriorly, but 3 long, strong and prominent caudal cirri present. The genus has the typical fronto-ventral and transverse arrangement of cirri with 3 strong anterior frontal cirri. The dorsal surface has several longitudinal rows of short cilia. Macronucleus in two parts each with an adjacent micronucleus. Several species have been recorded.

Undulating membrane is present in *Stylonychia* pattern. One right and one left row of marginal cirri, distinctly separate posteriorly. Presence of six dorsal kineties, caudal cirri present and often distinctly elongated, which have, however, a more slender and flexible body and intersecting undulating membranes There are two (two distinct segments of one macronucleus) macronuclei present which are oval in shape with single spherical to ovoid micronucleus each.

The genus *Stylonychia* has following species

S. pustulata Ehrenberg, 1838

S. mytilus Ehrenberg, 1838

S. putrina Stokes, 1885

S. notomorpha Stokes, 1885

Description of the *Stylonychia pustulata* -

The present species described as *Stylonychia pustulata* Ehrenberg (1830), (O. F. Muller, 1786) Ehrenberg, 1835 (O. F. Muller, 1786) Ehrenberg, 1838, S. pustulata (Deshmukh, 2010).Present author has found this species in fresh water ponds, ditches and streams, especially more abundantly in rain water bodies of Nashik district.

Body is inflexible and elliptical. It is measures about 44 to 125um in length and 22um to 81um in width. Both anterior and posterior ends are rounded and more or less parallel. Macronuclear segments two which are ovoid in shape. Each macronucleus is measures about 14 to 16um in length and 6 to 8um in width, lying left of the median. Micronuclei are two in number, they are small spherical, each in close contact with one of the macronuclear segments.

Contractile vacuole is single. It measures about 9um to 11um in length, present at the middle of the body. Endoplasm was seen bright yellow in colour in living condition. Food vacuoles are few to moderate in number. The large size food vacuole is measures about 27 to 32um in length.

Movement moderately slow, gliding, and often standing still for sometimes, rotation, around the longer axis on disturbance. The presences of various cirri are the organs of swimming and gliding locomotion. Marginal cirri are measures about 12 to 16um long. Right marginal row is composed of many more cirri than the left one. Three anterior frontal cirri enlarged, which measures 19-23 um in length. Four posterior cirri, arranged in an oblique hook-shaped row, and one enlarged buccal cirrus. Two postoral and three ventral cirri are present. Transverse cirri are five in number they measures about 20-24 um in length. The Caudal cirri are three in number; they are 16-25 um in length, very stiff and projecting laterally.

Comments

Body of present species is ovoid to reniform, inflexible and elliptical, with short dorsal bristles. Three long stiff caudal cirri are present and hence it is belongs to genus *Stylonychia*. This genus is first reported by Ehrenberg, 1830. Later many workers reported different species of this genus. Kudo (1966), Bick (1972), Berger & Foissner (1997), Lynn & Small (2000), reported the different species of the same genus. *Stylonychia pustulata* was identified by the shape of the body, the arrangement and the number of cortical elements, the mode of locomotion and morphological similarities in number and the positions of cirri. Movement moderately slow, gliding, and often standing still for sometimes, rotation, around the longer axis on disturbance.

Table 3: Comparison of different species of genus *Stylonychia* with present species, *S. pastulata*

Sr. No.	Particulars	*1*	*2*	*3*	*4*	*5*	*6*
		S. pustulata Ehrenberg, (1838)	*S. mytilus* Ehrenberg, (1838)	*S. putrina* Stokes, (1885)	*S. notomorpha* Stokes, (1885)	*S. pustulata* Deshmukh, (2010)	*S. pustulata* Present authors
1	Body shape	Ovoid	Elongate	Elongate	Elongate	Ovoid	Ovoid
2	Body dimensions	L- 150 um	L- 100 to 300um	L- 125 to 150um	L- 125um	L- 55 to 65um W- 30 to 38um	L-44 to 125um W- 22 to 81um
3	Macronuclei	2, ovoid	2	2, ovoid	2, ovoid	2, ovoid	2, ovoid
4	AZM	1/3 body length	1/3-1/2 body length	1/3-1/2 body length	1/3-1/2 body length	1/3 body length	1/3 of body length
5	Habitat	Fresh water	Fresh water	Fresh water	Fresh water	Muddy soil	Fresh water

AZM - Adoral zone of membranelle

Body of present species is ovoid and hence similar to previously described species *S. pustulata* but differs from *S. mytilus* (Ehrenberg, 1838) which is elongate and has anterior end wider than the posterior. *S. putrina* (Stokes, 1885 Deshmukh, 2010) is elongate and anterior end is not wider than posterior but anterior end is somewhat conical and rounded posterior end while in present species anterior end is rounded and posterior is broadly pointed and hence present species is also separates from *S. putrina.* It also distinguishes from *S. notomorpha* (Stokes, 1885) which has somewhat conically rounded anterior end and body laterally narrowed in some cases.

In body dimensions when compared with other species of *Stylonychia*, present species found to be small among all the previous ones (Table…..). Present species has 3 to 8 frontal, 2 post oral, 3 to 5 ventral, 5 transverse and 3 caudal cirri and hence similar to all the species of genus *Stylonychia* which also has 3 frontal, 3 ventral, 5 transverse and 3 caudal cirri.

AZM in present species also covers the one-third part the body length and hence this species is resembles that of *S. pustulata* but differs from *S. putrina* (Stokes, 1885), *S. mytilus* (Ehrenberg, 1838) in which AZM extends one-third to one-half the body length. It also separates from *S. notomorpha* (Stokes, 1885) in which AZM extends up to one-half the body length.

Present species has two ovoid macronuclei and hence similar to all the species of genus *Stylonychia.*

Present author compared present species with all the species of genus *Stylonychia* and found morphologically close similar to *S. pustulata* (Ehrenberg, 1838 in body outline, length of AZM and hence redescribed as a *Stylonychia pustulata.*

Figure - *Stylonychia pustulata*

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