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Diverse Freshwater Spirotrich Ciliate Fauna from Okhla Bird Sanctuary, Delhi, India

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ABSTRACT

India, with 2.4% of the world's area, has over 8% of the world's total biodiversity, making it one of the 12 mega-diversity countries in the world. This status is based on the species richness and levels of endemism recorded in a wide range of taxa of both plants and animals. In the present study, we explored ciliate diversity from fresh water samples collected from Okhla Bird Sanctuary (28°32'43.5"N, 77°18'41.7"E). It is a bird sanctuary at the Okhla barrage over Yamuna River. The most prominent feature of the sanctuary is the large lake created by damming the river. Cells isolated from the water samples collected from the lake were cultured in vitro under laboratory conditions. Identification and characterization of ciliates was done in vivo by Phase Contrast Microscopy, Feulgen staining and silver impregnations. In total, 27 species from 24 genera were found. A higher proportion of Spirotrichean (44.45%; 10 genera, 12 species) species were identified. Other species belonging to Classes Karyorelictea, Litostomatea, Prostomatea, Phyllopharyngea and Colpodea were also found but relatively in lesser proportion.

KEYWORDS : Ciliates, Okhla Bird Sanctuary, Biodiversity

Introduction

India is one of the richest countries in the world in terms of biodiversity. The country is estimated to have more than 7% of the world's flora and 6.5% of fauna. Adding to this is a very high diversity of human-influenced ecosystems, including agricultural and pasture lands, and a diversity of domesticated plants and animals. India is also considered one of the world's eight centers of origin of cultivated plants. Being a predominantly agricultural country, India also has a mix of wild and cultivated habitats, giving rise to very specialized biodiversity, which is specific to the confluence of two or more habitats (MoEF & Kalpavriksh, 2004).

The Phylum Ciliophora is a well-defined, monophyletic protist taxon, which clusters together with the Dinoflagellata and the Apicomplexa to form the clade Alveolata (Michaela, Struder-Kypke & Lynn, 2010). Ciliates are now divided into two subphyla and 11 classes (Lynn, 2008) They are found in variety of micro-habitats and play an important ecological role, often functioning as major trophic links in food webs (Lynn, 2008). Despite this importance, our knowledge about ciliate diversity is far from complete. Here, we describe the diverse spirotrich ciliates found in a fresh water body located in Delhi, India.

Materials and Methods

Collection of ciliates

Ciliates were collected from Okhla Bird Sanctuary in Delhi (Figure 1). Nytex nets of decreasing mesh sizes were used in succession to filter out large crustaceans, debris and other unwanted materials. Several liters of water samples were strained through mesh of size < 50microns and the concentrate containing ciliate fauna was transferred to labeled containers. The collections were transferred to large troughs in the laboratory. Mixed planktonic cultures were grown at room temperature and examined for the presence of ciliates. Such water samples were subjected to periodic microscopic examination for about 20 days. Single cells were isolated to raise clonal cultures (Arora, Gupta, Kamra & Sapra, 1999).



Figure-1: Collection site and map

In-vitro culturing of ciliates

Ciliates were cultured in Pringsheim's medium (Chapman-Andresen, 1958). Boiled cabbage was added to the medium to promote the growth of bacteria which served as the primary food source for the ciliates. The cultures were sub-cultured after every 4 days.

Morphological Studies

The live specimens were observed under a high-power oil-immersion objective with bright field, phase contrast and were complemented with protargol staining (Kamra & Sapra, 1990) and Feulgen staining (Chieco and Derenzini, 1999; Feulgen, 1914). Identification, nomenclature and terminology of ciliate species was done according to Berger (1999), Foissner (1998), Corliss (1979), Lynn and Small (2000), Lynn (2008).

Results and discussion

A total of 12 species belonging to 10 different genera were identified representing two major subclasses of the class spirotrichea. Class Spiro-

trichea Bütschli, 1889 is a diverse assemblage of ciliates that are cosmopolitan. The class name arised from the characteristically spiraling nature of the adoral zone of membranelles or oral polykinetids, which emerge from the oral cavity and spiral in counter-clockwise direction as they wrap around the anterior body surface. The class is divided into seven subclasses. They locomote typically using somatic polykinetids, called cirri, although several included groups have simpler somatic kinetids. Molecular biological research on the developing macronuclei of spirotrichs demonstrated the presence of polytene chromosomes and the ultimate differentiation of macronuclear DNA characterized by gene sized pieces. Subclass stichotrich and hypotrichs have been of particular interest to biologists interested in the development of pattern in cells. Stichotrichs have considerable regenerative powers, which have also made them useful models for developmental biologists.

The present study shows that ciliates belonging to class Spirotrichea were most abundant and those belonging to classes Karyorelictea, Prostomatea, Colpodea and Phyllopharyngea were least abundant in Okhla Bird Sanctuary (Table 1).

Class Spirotrichea Bütschli, 1889
Sub class Hypotrichia Stein, 1859
Order Euplotida Small & Lynn, 1985
Sub order Euplotina Jankowski, 1979
Family Euplotidae Ehrenberg, 1838
Genus *Euplotes* Ehrenberg in Hemprich & Ehrenberg, 1831

1. *Euplotes* sp. 1

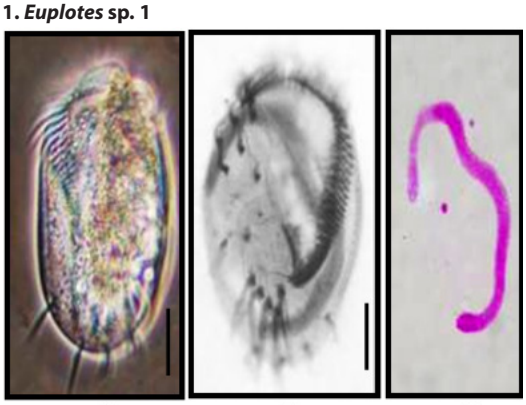


Figure-2: *Euplotes* sp. 1, from life (a), after Protargol staining (b) and Feulgen staining (c). Scale bar represents 20 µm.

Diagnostic features: Size (in life): 70-100 x 60-85 µm. Body ovoid, rigid, dorso-ventrally flattened with very large AZM usually extending at least two-thirds the body length, 48-52 membranelles, C-shaped macronucleus with an arched back, 2 micronuclei. 9 Fronto-ventral cirri, 5 transverse cirri, 4 caudal cirri and 10 dorsal kineties. Presence of dorsal and ventral argysome which is a diagnostic feature in species identification.

2. *Euplotes* sp. 2

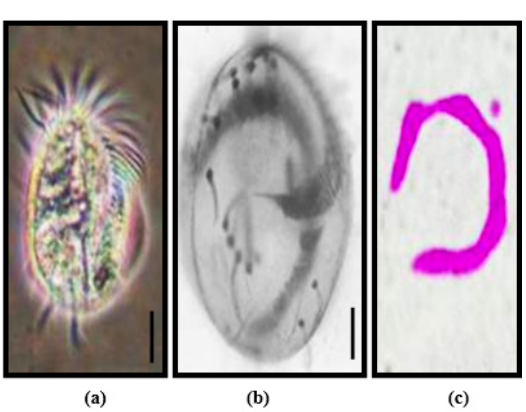


Figure-3: *Euplotes* sp. 2, from life (a), after Protargol staining (b) and Feulgen staining (c). Scale bar represents 20 µm.

Diagnostic features: Size (in life): 60-80 x 50-60 µm. Body ovoid, rigid, dorso-ventrally flattened with AZM usually extending <50% of the body length, 30-32 membranelles C-shaped macronucleus, 1 micronucleus. 9 fronto-ventral, 5 tranverse, 4-5 caudal cirri and 8 dorsal kineties.

3. *Euplotes* sp. 3.

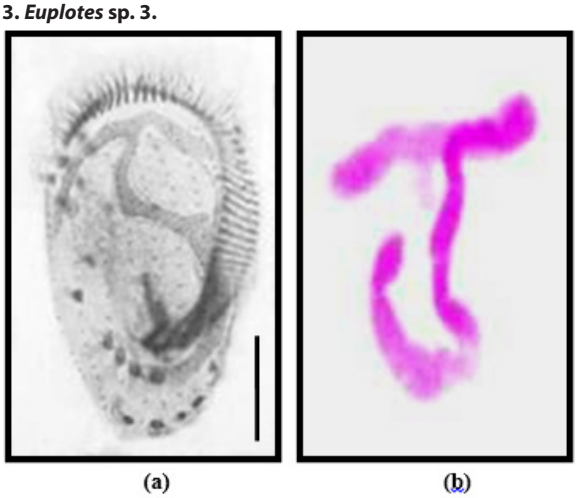


Figure-4: *Euplotes* sp. 3, after Protargol staining (a), and Feulgen staining (b). Scale bar represents 20 µm.

Diagnostic features: Size (in life): 60-80 x 50-60 µm. Body ovoid, rigid, dorso-ventrally flattened with AZM very large usually extending at least two third of the body length, 48-50 membranelles, J-shaped macronucleus, 1-2 micronuclei. 9 fronto-ventral cirri, 5 transverse, 4 caudal cirri and 10 dorsal kineties.

Family Aspidiscidae Ehrenberg, 1830
Genus *Aspidisca* Ehrenberg, 1830

4. *Aspidisca* sp.

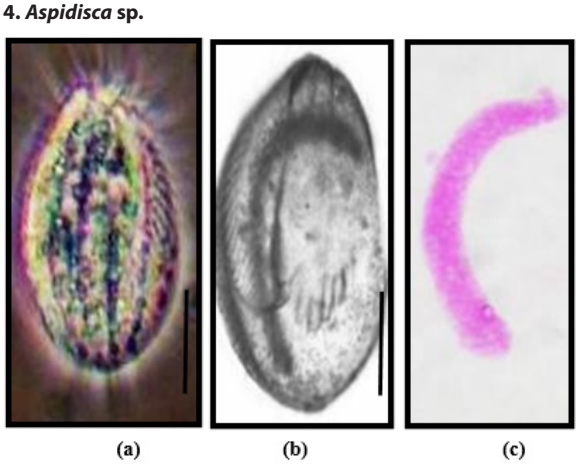


Figure-5: *Aspidisca* sp., from life (a), after Protargol staining (b) and Feulgen staining (c). Scale bar represents 20 µm.

Diagnostic features: Size (in life): 40-60 x 20-30 µm. Oval and rigid body with dorsal ridges. Body tends to be more convex on the right than on the left. The adoral zone of membranelles (AZM) is in two parts separated on the left margin of the body. The posterior part is a series of membranelles lining the peristome while the anterior part is comprised of 2-8 cilia. No marginal and caudal cirri, paroral absent, 7 frontoventral (6 frontal and 1 ventral) cirri and 7 transverse cirri. Macronucleus horseshoe-shaped, 1-2 micronuclei.

Subclass Stichotrichea Small & Lynn, 1985

Order Sporadotrichida Fauré-Fremiet, 1961
Family Oxytrichidae Ehrenberg, 1830
Genus *Oxytricha* Bory de St. Vincent in Lamouroux, Bory de St. Vincent & Deslongchamps, 1824

5. *Oxytricha* sp.

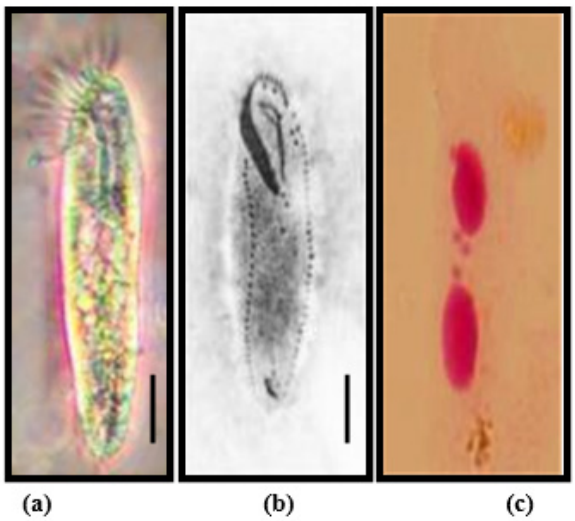


Figure-6: *Oxytricha* sp., from life (a), after Protargol staining (b) and Feulgen staining (c). Scale bar represents 20 µm.

Diagnostic features: Size (in life): 90-130 x 25-35 µm. Flexible body, cortical granules present, AZM usually covers <40% of body length, undulating membranes (paroral and endoral) in *Oxytricha* pattern (paroral and endoral membranes are slightly curved and optically intersect at the middle and the buccal cavity is usually flat and narrow), 18 frontoventral-transverse (FVT) cirri which are arranged in V-shaped pattern. Postoral ventral cirri in dense cluster behind buccal vertex, 2 pretransverse cirri and 5 transverse cirri, oone left and one right marginal cirral (LMC & RMC) row , 6 dorsal kineties (DKs), 2 macro- and 2-4 micronuclei. Caudal cirri present.

Genus *Notohymena*, Blatterer and Foissner, 1988

6. *Notohymena* sp.

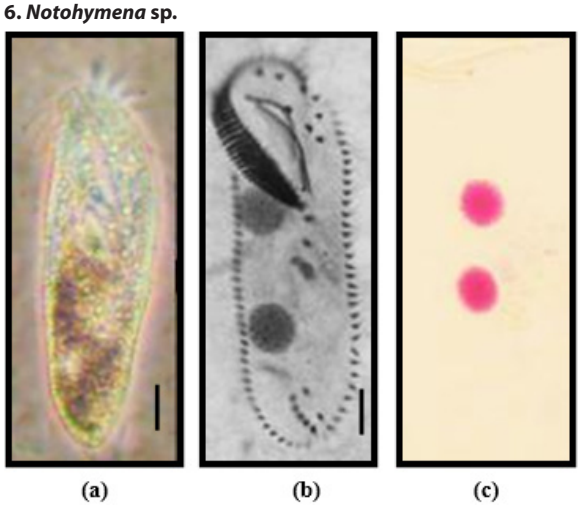


Figure-7: *Notohymena* sp. 1, from life (a), after Protargol staining (b) and Feulgen staining (c). Scale bar represents 20 µm.

Diagnostic features: Size (in life): 150-160 x 40-50 µm. Flexible, yyellowish-green cortical granules arranged in clusters, AZM covering 1/3rd of the body length and consists of 36 adoral membranelles, 18 FVT cirri, one RMC and one LMC which are almost confluent posteriorly, 6 dorsal rows, 7 caudal cirri , 2 macronuclei and amicronucleate (no micronucleus).

Genus *Paraurostyla* Borror, 1972

7. *Paraurostyla coronata*

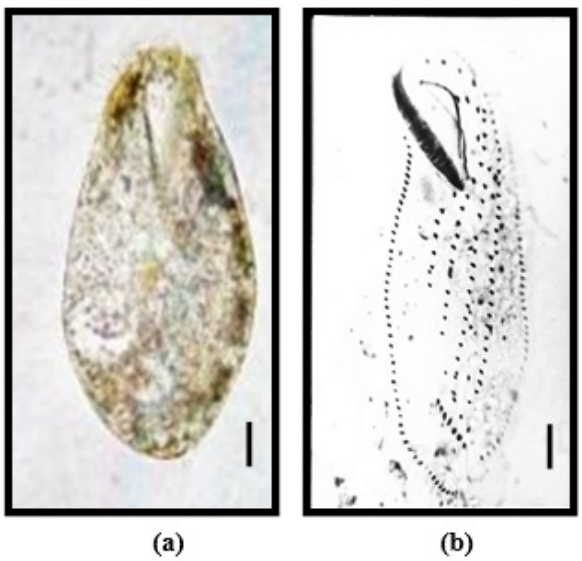


Figure-8: *Paraurostyla coronata*, from life (a) and after Protargol staining (b). Scale bar represents 20 µm

Diagnostic features: Size (in life): 170-200 x 50-80 µm. Body flexible, dorsoventrally flattened and exhibits diffused green cytoplasmic pigmentation with pink coloration at the anterior and posterior segments. AZM one third of the body length and contains 65-78 adoral membranelles, 7 frontal cirri are arranged in a distinct pattern where 6 of them are placed in a single continuous row away from the buccal cirrus, 6-8 longitudinal rows of ventral cirri, one LMC and one RMC, 6-10 transverse cirri arranged in an oblique pattern, 6 dorsal kineties, 15-18 caudal cirri arranged in 3 rows of 5-6 cirri in each row, 2 macronuclei and 4 micronuclei.

Genus *Gastrostyla* Engelmann, 1862

8. *Gastrostyla* sp.

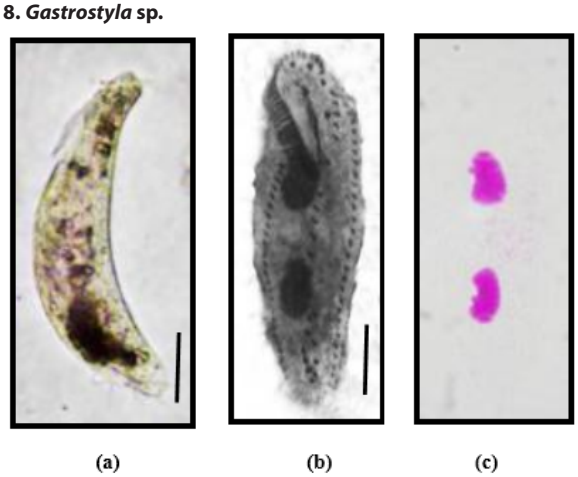


Figure-9: *Gastrostyla* sp., from life (a), after Protargol staining (b) and Feulgen staining (c). Scale bar represents 20 µm.

Diagnostic features: Size (in life): 80-100 x 40-50 µm. Body flexible, dorsoventrally flattened, cytoplasm colorless, AZM question mark shaped and covers almost 40% of the body length. Undulating membranes (UM) in *Oxytricha* pattern. Number of frontoventral and postoral ventral cirri is distinctly more than 7 and they form a continuous, slightly oblique frontoventral row. 2 pretransverse, 5 transverse cirri,

one RMC and one LMC, 5-6 dorsal kineties and caudal cirri present.

Genus *Tetmemena* Eigner, 1999

9. *Tetmemena* sp.

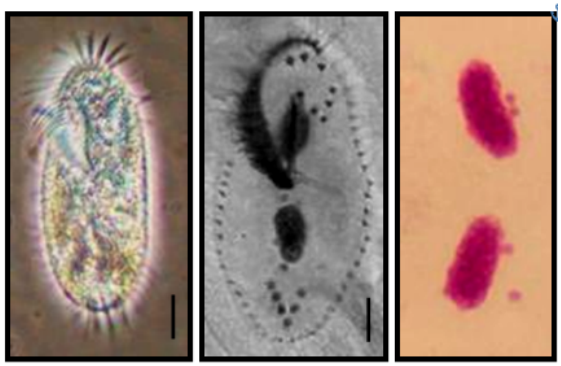


Figure-10: *Tetmemena* sp., from life (a), after Protargol staining (b) and Feulgen staining (c). Scale bar represents 20 µm.

Diagnostic features: Size (in life): 90-130 x 30-60 µm. Body rigid, dorsoventrally flattened with body length to width ratio of 2:1. AZM covers >40% of the body length and made up of 34-39 adoral membranelles, 18 FVT cirri, one RMC and one LMC, caudal cirri present, 2 macronuclei, 2-4 micronuclei.

Genus *Laurenteilla* Dragesco & Njiné, 1971

10. *Laurentiella* sp.

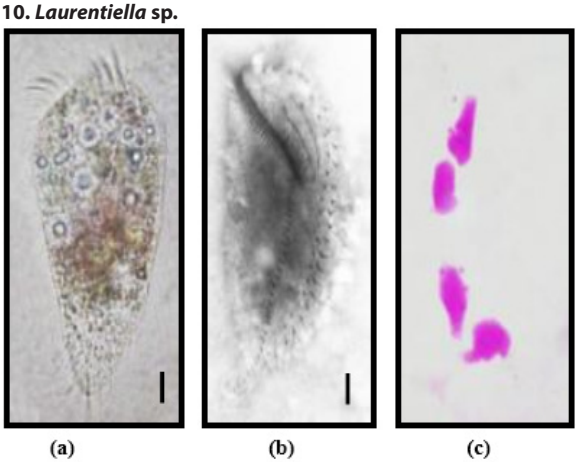


Figure-11: *Laurentiella* sp., from life (a), after Protargol staining (b) and Feulgen staining (c). Scale bar represents 20 µm.

Diagnostic features: Size (in life): 200-220 x 90-100 µm. Body rigid, anterior end broad and pointed posterior end, AZM 50% of body length, UM in *Stylonychia* pattern (paroral and endoral are straight or very slightly curved and arranged almost in parallel and the buccal cavity is wide and flat), one RMC and one RMC, transverse cirri usually 5-7 in number, 3 caudal cirri, 2 midventral rows, 4 macronuclear nodule and 6-7 micronuclei.

Order Urostylida Jankowski, 1979
Family Pseudourostylidae Jankowski, 1979
Genus *Pseudourostyla* Borror, 1972

11. *Pseudourostyla cristata* (Borror, 1972)
Syn: *Urostyla cristata* Jerka-Dziadosz, 1964

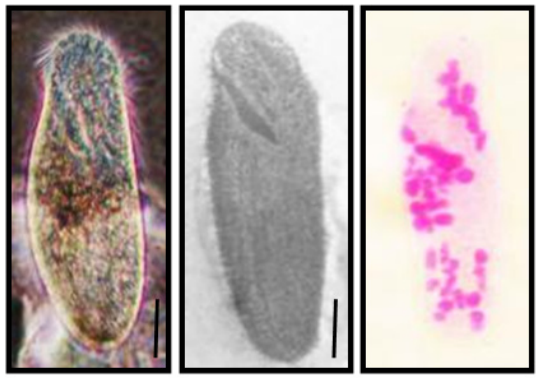


Figure-12: *Pseudourostyla cristata*, from life (a), after Protargol staining (b) and Feulgen staining (c). Scale bar represents 50µm.

Diagnostic features: Size (in life): 250-300 x 60-80 µm. Body flexible, brownish granules, dorso-ventrally flattened with body length width ratio as 3.2:1, body ellipsoidal with both ends broadly rounded, extrusomes present, AZM covers about 40% of body length and contains 88 adoral membranelles, UM straight, 4-5 LMC and RMC, numerous macronuclear nodules scattered throughout the cytoplasm.

Family Urostylidae Bütschli, 1889
Genus *Urostyla* Ehrenberg, 1830

12. *Urostyla grandis* (Ehrenberg, 1830)
Syn: *Metaurostyla polonica* Jankowski, 1979 *Urostyla chlorelligera* Foissner, 1980

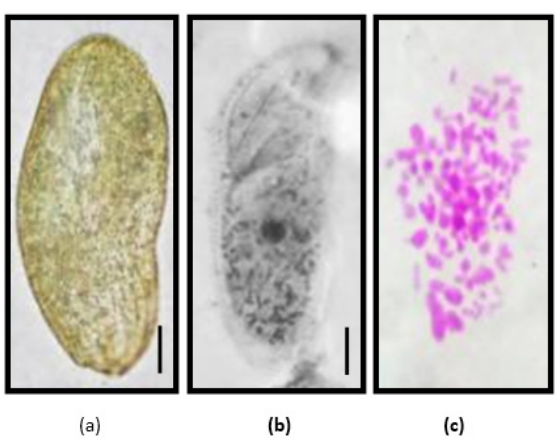


Figure-13: *Urostyla grandis*, from life (a), after Protargol staining (b) and Feulgen staining (c). Scale bar represents 50µm.

Diagnostic features: Size (in life): 300-400 x 90-120 µm. Body flexible, dorsoventrally flattened, ellipsoidal, both ends broadly rounded, body tends to be more convex on the left than on the right, body length to width ratio is 3:1, yellow-greenish cortical granules arranged in longitudinal rows on dorsal and ventral side, AZM one-third of the body length and contains 47-60 adoral membranelles, UM long and curved, optically intersecting in mid-region, 3-4 midventral rows 11 transverse cirri arranged in slightly curved oblique rows, 4 RMC and LMC, numerous (100-150) macronuclear nodules scattered throughout cytoplasm.

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Table-1: *Spirotrich Ciliate* diversity at Okhla Bird Sanctuary, Delhi

Class	Genus	Number of Species Found
Spirotrichea	*Euplotes*	3
	Aspidisca	1
	Oxytricha	1
	Notohymena	1
	Paraurostyla	1
	Gastrostyla	1
	Tetmemena	1
	Laurentiella	1
	Pseudourostyla	1
	Urostyla	1

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