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NEW RECORDS OF SOME HAEMOPARASITES AFFECTING *PUNTIUS TICTO* (PISCES: CYPRINIDAE) IN INDIA: OBSERVATIONS ON INTERACTION AND DISPLACEMENT OF SPECIES

N. GUPTA[1], D.K. GUPTA[2] & P. YADAV[2]

[1]*Department of Animal Science, Rohilkhand University, Kanoongoxan 188, Bareilly-243006, U.P., India*

[2]*Post Graduate Department of Zoology, Bareilly College, Bareilly, U.P., India*

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ABSTRACT: Haematocrit examination of *Puntius ticto* revealed 5% *Trypanosoma ticti* n. sp. and 4% *Trypanoplasma cyprinoides* n. sp. infection under lone conditions of infectivity, whereas under concomitant conditions, the parasite density of *Trypanosoma ticti* n. sp. fell sharply in the infected samples. The permanent preparations of the parasites were morphotaxonomically compared with the previously described forms and discussed. Cross transmission tests were performed to identify their status and the results indicate the occurrence of new records. Based on the above observations, inferences were made on interaction and displacement of the flagellate species recorded, whereby the trypanoplasm species displaced the trypanosome species under concomitant conditions (natural and experimental infections). This may be because the bulkier consistency and greater metabolic requirements of the trypanoplasm species accounts for greater consumption of food resources.

KEY WORDS: Haemoparasites, *Trypanosoma ticti* n. sp., *Trypanoplasma cyprinoides* n. sp., Pisces, Cyprinidae, *Puntius ticto*, description, interaction, displacement.

INTRODUCTION

There has been an increasing expansion in aquacultural practices during the past few decades and stress is being laid on fish pathological studies. Reports on blood flagellates inhabiting freshwater fish have sprung up from different corners of the globe. Monoflagellates of the genus *Trypanosoma* Gruby, 1841 (KHAN, 1977; GUPTA & GUPTA, 1988; LOPES, PAULA-LOPES & RIBEIRO, 1991; HSU *et al.*, 1995) and biflagellates of the genus *Trypanoplasma* Laveran et Mesnil, 1901 (WAHUL, 1985; GUPTA & GUPTA, 1987; KRUSE, STEINHAGEN & KORTING, 1989) have been reported from different fish hosts: However, concomitant infection has rarely been reported (KHAN & LACEY, 1986; GUPTA & GUPTA, 1990).

The existence of two or more species in a particular microhabitat is important ecologically and affects the population density of the competing species. Flagellates infecting fish of the genus *Puntius* have already been recorded (WAHUL, 1986a, b). The present communication is an attempt to assess differential and concomitant infectivity of two new mastigophorean species of the genera *Trypanosoma* and *Trypanoplasma* in a cyprinid fish, *Puntius ticto* and to make inferences on interaction and displacement of the species in the micro niche, blood under natural and experimental conditions of infectivity.

MATERIAL AND METHODS

Hosts and parasites: Fish were collected from Nekpur pond of Bareilly district, India and maintained in the laboratory aquaria under constant observation. The methods of smear preparation and calculation of indices were as described earlier (GUPTA, 1986). Examinations were made under a phase contrast microscope using oil immersion lens.

Cross transmission tests: It was ensured that the recipient fish were infection-free by examining them twice a week for 30 days before inoculation. One ml of cardiac blood was collected in a heparinized syringe from the infected donors, pooled and the inoculum prepared as given earlier (GUPTA & GUPTA, 1988). The recipient fish (maintained in glass aquaria under favourable conditions of temperature, food and O_2) were inoculated intraperitoneally with 0.25 ml inoculum/fish.

DESCRIPTION OF SPECIES

The blood of *Puntius ticto* was infected with a polymorphic *Trypanosoma* species (small, intermediate and large - increasing order of abundance) and a dimorphic *Trypanoplasma* species (stumpy and bulky). The incidence was rather meagre (*Trypanosoma* species: 5%; *Trypanoplasma* species: 4%) and concomitant infection was evident in 6 samples where the trypanoplasms (10-25 forms/20 mins scan) outnumbered the trypanosomes (4-6 forms/20 mins scan). However, under lone infectivity, the trypanosomes (10-100 forms/20 mins scan) had a higher parasite density as compared to the biflagellates (10-25 forms/20 mins scan).

Trypanosoma ticti n. sp.

Morphology

Shape: The body is elongated and pointed in shape and the curvature of the body is proportional to the cell

body length. The parasite is sinuous when alive; upon fixation, it takes a S, C or even a compressed form.

Cytoplasm: The cytoplasm takes a light stain; granules are more prominent in the posterior half of the body, being more concentrated towards the margins.

Nucleus: The nucleus may be spherical but is generally elongated or bean-shaped. It takes a deep stain and may occupy the entire width of the body. Karyosome was not observed in any of the specimens.

Kinetoplast: The kinetoplast is sub-terminal in all the forms and its extremity extends posteriorly in a clear beak-like fashion. The small and intermediate forms possess a spherical kinetoplast whereas in the larger forms, the organelle may even be elongated and placed either parallel to the body length or transversely.

Flagellum and undulating membrane: The flagellum arises from the kinetoplast, borders a poorly-developed undulating membrane in the intermediate and large forms and finally extends anteriorly as a free flagellum.

Measurements: The measurements of all the forms with their range and mean values are given in Table 1.

Indices: Nuclear index: 0.66; Kinetoplast index: 12.28; Flagellar index: 3.7.

Discussion

Trypanosomes have been well reported from cyprinid fishes. An unnamed trypanosome was first recorded from *Puntius carnaticus* by LINGARD (1904). BRUMPT (1906) reported *T. barbi* from *Puntius fluviatilis*. JOSHI (1976, 1978) reported *T. mrigali* from *Cirrhinus mrigala*, *T. bata* from *Labeo bata* and *T. stigmai* from *P. stigma*. *T. baigulensis* was recorded from *C. reba* by PANDEY & PANDEY (1974). *T. monomorpha* was described by GUPTA & JAIRAJPURI (1985) from *Catla catla*. WAHUL (1986b) recorded three new species of trypanosomes from the genus *Puntius*: *T. puntii* from *P. kolus*, *T. rayi* from *P. sarana* and *T. marathwadensis* from *P. hexastichus*.

All the species reported from cyprinid fishes are either monomorphic or dimorphic (a feature which differentiates the present form from them) except *T. mrigali*, in which the author reported polymorphism and mentioned 3 morphologically varied forms: stumpy, rectangular and common forms varying in length from 21.4 to 37.2 µm. The present species assumes a much greater length, reaching 63 µm in length and being narrower, never reaching 7.8 µm as in the rectangular form of *T. mrigali*. Significant variations also exist in the cell body length, nuclear dimensions, cytomorphological characteristics and nuclear index. Hence the species under discussion cannot be synonymized with *T. mrigali*.

Cross transmission tests (Series A)

Cross transmission tests were performed to investigate the taxonomical status of the discovered trypanosome. Cyprinid fish (*Cirrihinus mrigala*) in which polymorphic trypanosomes have been reported were chosen as the test models and the fish were accordingly divided into 3 groups: Group A: *Puntius ticto* (control); Group B: *Puntius ticto* (experimental); Group C: *Cirrhinus mrigala* (experimental). Each group contained 10 samples. Fishes of Group B and C were inoculated with 0.25 ml of *Trypanosoma*-infected blood and examinations were

Component parts of the parasite*	*Trypanosoma ticti* n. sp.			*Trypanoplasma cyprinoides* n. sp.	
	Small	Intermediate	Large	Stumpy	Bulky
CF	22.2 (20.0-25.0)	40.3 (35.0-47.0)	58.7 (53.0-63.0)	–	–
CL	14.1 (12.0-16.0)	34.6 (25.0-40.0)	43.0 (42.0-50.5)	30.6 (20.0-39.0)	50.1 (45.0-58.0)
CB	2.1 (2.0-2.5)	2.4 (2.0-3.0)	5.25 (4.2-6.0)	10.0 (6.0-16.0)	11.3 (10.5-16.0)
AF	7.1 (6.0-8.0)	8.8 (7.0-10.5)	15.5 (13.0-21.0)	–	15.9 (13.0-23.0)
PF	–	–	–	–	10.6 (8.0-14.0)
NL	2.8 (2.0-3.5)	3.2 (2.2-4.5)	5.8 (4.0-7.3)	6.8 (4.5-10.0)	8.05 (2.4-19.0)
NB	1.5 (1.0-2.0)	2.4 (1.5-2.8)	3.4 (3.0-5.0)	3.2 (2.0-5.0)	4.6 (2.3-8.0)
KL	0.7 (0.5-1.0)	0.9 (0.5-1.0)	1.27 (0.9-1.7)	4.7 (2.5-8.0)	6.6 (4.0-10.0)
KB	–	–	–	1.3 (1.0-2.0)	1.59 (1.2-2.2)

Table 1.– Measurements of *Trypanosoma ticti* n. sp. from *Puntius ticto*. All values are in µm. Figures in parentheses give the range values. AF = anterior flagellum; CB = breadth of cell body; CF = total length; CL = length of cell body; KB = kinetoplast breadth; KL = kinetoplast length; NB = nuclear breadth; NL = nuclear length; PF = posterior flagellum.

Group	Parasite	Host	Duration (days)																			Parasitaemia (No. of parasites/20 mins. scan after 21 days)	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Donor	Experimental
SERIES A	*Trypanosoma*																								
A	*T. ticti* n. sp.	*Puntius ticto* (Control)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B	–do–	*Puntius ticto* (Experimental)	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	10-100	10-60
C	–do–	*Cirrhinus mrigala* (Experimental)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SERIES B	*Tryplanoplasma*																								
D	*T. cyprinoides* n. sp.	*Puntius ticto* (Control)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E	-do–	*Puntius ticto* (Experimental)	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	10-25	6-15
F	–do–	*Puntius sarana* (Experimental)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 2.– Effect of cross transmission of *Trypanosoma ticti* n. sp. and *Trypanoplasma cyprinoides* n. sp. (0.25 ml inoculum/fish). The values are for 10 observations for each group.

made every 24 h for 21 days to detect infection. It was observed that the trypanosomes appeared in the blood circulation of Group B fish at day 3 p.i. (post inoculation) whereas they failed to establish themselves in Group C even up to 21 days p.i. (Table 2), thereby demonstating their specificity for *Puntius ticto*.

Conclusion

The above examinations on morpho-taxonomical, cytomorphological and cross transmission tests infer that the polymorphic trypanosome discovered from the blood of *Puntius ticto* is a new species and the name *Trypanosoma ticti* n. sp. is proposed to accomodate the new species.

Trypanoplasma cyprinoides n. sp.

Morphology

Shape: The shape of the body may be stumpy (Fig. 1B) or bulky (Fig. 1C), a feature on which the morphological forms have been named. The extremities of the body are blunt and rounded.

Cytoplasm: The cytoplasm is vacuolated and granular. The granules are usually uniformly distributed but may be more concentrated towards one margin. Vacuoles are generally scattered.

Nucleus: The uniformly stained, dorso-anteriorly situated nucleus occupies the broad end of the cell body. The shape is elongated, bean-shaped or rarely somewhat conical, protruding conspicuously from the dorsal body surface.

Kinetoplast: The kinetoplast is situated opposite to the nucleus lying along the curved, ventral side but may also be oriented differentially. The anterior end may be narrower or the organelle may be more or less uniform in shape.

Flagella and undulating membrane: The kinetosomes were not observed in any of the forms. The flagella appear to originate approximately from the kinetoplast. The anterior flagellum is always longer than the posterior one. The undulating membrane is not well marked and is bordered by the posterior flagellum.

Measurements: The statistical data of the stumpy and bulky forms are given in Table 1.

Discussion

The biflagellates recorded from cyprinid fishes are *T. saranae*, *T. lomi* and *T. solapurensis* from *Puntius sarana*, *P. hexastichus* and *P. jerdoni* respectively (WAHUL, 1986a). Interestingly, all the species reported are monomorphic, whereas the form under consideration is definitely dimorphic, existing clearly as stumpy and bulky forms, and may reach a body length of a maximum of 58 µm, the largest dimorphic trypanoplasm recorded from the freshwater fishes of India. Therefore, the present form can be marked off from these and other monomorphic and polymorphic species due to its distinct dimorphism.

From India, *T. atti* (JOSHI, 1982), *T. qadrii* (KRISHNAMURTHY & WAHUL, 1986) and *T. maguri* (GUPTA & GUPTA, 1987) have been reported as dimorphic species, as is also the species under discussion. JOSHI (1982), in his description of *T. atti* from *Wallago attu*, reported long and ovoid forms of the parasite. However, the present form

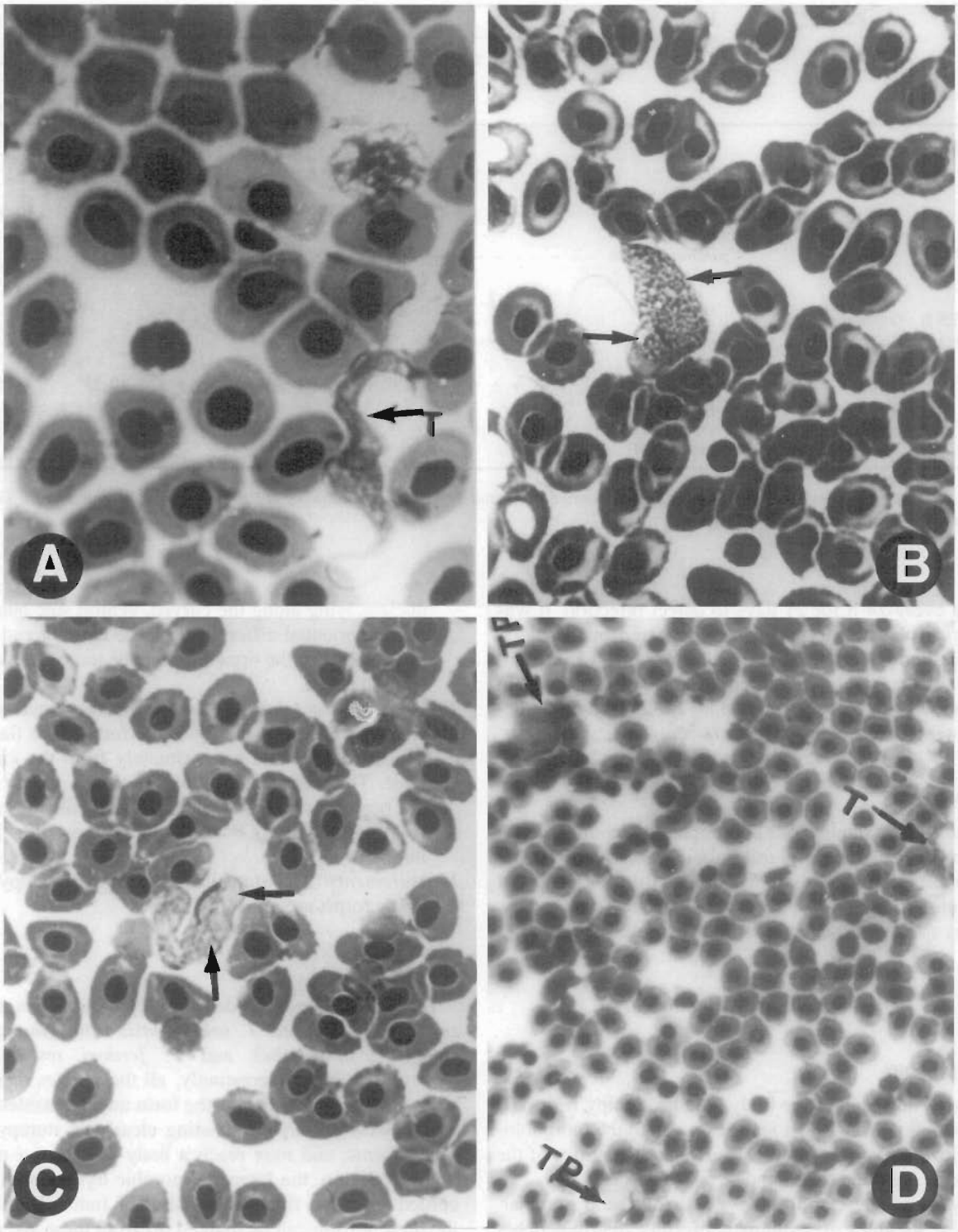

Fig. 1.– Photomicrographs of haemoparasites infecting *Puntius ticto*: A) *Trypanosoma ticti* n. sp. (x 1500); B) *Trypanoplasma cyprinoides* n. sp., stumpy form (x 1000); C) *Trypanoplasma cyprinoides* n. sp., bulky form (x 1000); D) concomitant infection with *Trypanosoma ticti* n. sp. (T) and *Trypanoplasma cyprinoides* n. sp. (TP) (x 400).

can be differentiated from *T. atti* in the body shape and size, flagellar lengths and nuclear dimensions. The present form is also contrasted from *T. qadrii* by its vastly larger body size and nuclear dimensions. The present form is further differentiated from *T. maguri* in its distinctly broader body, shorter flagella and broader nucleus.

Cross transmission tests (Series B)

The biflagellated parasites from *Puntius ticto* were transmitted to the same fish host as well as *P. sarana*, the other cyprinid fish from which trypanoplasms have been recorded. The fish were characterized as: Group D: *P. ticto* (control); Group E: *P. ticto* (experimental); Group F: *P. sarana*. The inoculum of *Trypanoplasma*-infected blood was prepared as stated earlier. Fishes of Group E and F were inoculated intraperitoneally with 0.25 ml inoculum/fish. Examinations every 24 h for 21 consecutive days failed to demonstrate parasites in Group F fish, but they appeared in the circulation of Group E fish on day 4 p.i. (Table 2), proving their specificity for *Puntius ticto*.

Conclusion

In the light of the above observations and cross transmission tests, the trypanoplasm discovered from the blood of *P. ticto* is considered to be new and the name *Trypanoplasma cyprinoides* n. sp. is proposed to designate the new species.

SPECIES INTERACTION

Cross transmission tests (Series C)

The third set of cross transmission tests was performed to observe the phenomenon of interaction of blood flagellates in the blood milieu of their vertebrate hosts. For this purpose, *P. ticto* were divided into three groups: Group A: trypanosome-infected; Group B: trypanoplasm-infected; Group C: experimental trypanosome-infected fish (recipient) to be inoculated with trypanoplasm-infected blood. The parasitaemia in all the groups was recorded on day 1 of experimentation and Group C fish were inoculated with 0.25 ml of trypanoplasm-infected blood (density: 10-25 forms/20 mins scan) which were already parasitized with trypanosomes at their natural density. The fishes were kept under constant observation and on day 21 p.i., the trypanosome population in Group C dwindled down to 10-18 forms/20 mins scan, but the trypanoplasm population remained almost unaffected (Table 3).

The population densities of the flagellates may thus be represented as:

A) natural:
- lone *Trypanosoma ticti* n. sp. infections: 10-100 forms/20 mins scan;
- lone *Trypanoplasma cyprinoides* n. sp. infections: 10-25 forms/20 mins scan;
- concomitant infection: *Trypanosoma ticti* n. sp.: 4-6 forms/20 mins scan; *Trypanoplasma cyprinoides* n. sp.: 10-25 forms/20 mins scan;

B) experimental:
- concomitant (day 21 p.i.): *Trypanosoma ticti* n. sp.: 10-18 forms/20 mins scan; *Trypanoplasma cyprinoides* n. sp.: 10-20 forms/20 mins scan.

The existence of two or more species in a particular microhabitat is important ecologically and may affect the population density of the competing species. The interaction between species may influence either, both or neither population (specially under abundant food resource conditions). PONTIN (1982) postulated three possible alternatives for interspecific competition: species A displaces species B; instability with displacement of either by the other depending upon starting density or other factors; and stable co-existence.

The values obtained during the present studies indicate that under natural infectivity, the population density of *Trypanosoma ticti* n. sp. fell considerably below concomitant conditions whereas that of *Trypanoplasma cypri-*

Group	Parasitaemia (no. of parasites/20 mins scan)			
	Day 1		Day 21	
	Trypanosoma	*Trypanoplasma*	*Trypanosoma*	*Trypanoplasma*
SERIES C				
A (Trypanosome-infected *Puntius ticto*)	10-100	–	10-80	–
B (Trypanoplasm-infected *Puntius ticto*)	–	10-25	–	10-20
C (Inoculation of trypanoplasm-infected inoculum into trypanosome-infected hosts)	10-100	10-25 ↑ Inoculum	10-18	10-20

Table 3.– Effect of cross transmission of trypanoplasm-infected blood (0.25 ml inoculum/fish) in trypanosome-infected hosts. The values are for 10 observations for each group.

noides n. sp. remained unaffected. The cross transmission tests (series C) provide a further justification of the fact, where the trypanoplasm population suppressed the trypanosome population as observed at day 21 p.i. It would appear that the biflagellate species dominates as it maintained its density under concomitant conditions, whereas the monoflagellate population fell considerably. Although illuminating laboratory studies have been conducted in other ecological groups (KHAN & LACEY, 1986), similar observations on the population densities of flagellate parasites of fish blood have yet to be demonstrated. GUPTA & GUPTA (1990) reported concomitant infection in *Mystus vittatus* with *Trypanosoma vittati* and *Trypanoplasma tengari*, but no attempt was made to provide justification of competition and interaction amongst the species.

GUPTA & GUPTA (1987) observed that hypoglycemia due to *Trypanoplasma* was more severe (57% glucose fall) than due to *Trypanosoma* (37% fall) in *Clarias batrachus*. Both the species appear to use the same resources (blood) and apparently the metabolic requirements of the biflagellates are greater due to their voluminous size. Consequently, this parasite attempts to exclude *Trypanosoma* from the blood which it was otherwise occupying under lone infectivity. It would appear that species A (*Trypanoplasma*) is displacing species B (*Trypanosoma*) under concomitant conditions (natural as well as experimental) thereby causing a decrease in parasitaemia of *Trypanosoma*. The present studies are not conclusive and further work is in progress to cultivate the parasites *in vitro* and to provide further explanation for the changes in parasite density under concomitant manifestations.

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