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Predatory Activity of *Psychoda alternata* Say (Diptera: Psychodidae) Larvae on *Biomphalaria glabrata* and *Lymnaea natalensis* Snails and the Free- Living Larval Stages of *Schistosoma mansoni*

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Abstract: Larvae of *Psychoda alternata* Say (drain fly) proved experimentally to have different degrees of predatory activity on egg masses of *Biomphalaria glabrata* Say, and *Lymnaea natalensis* Kruss, the snail hosts of *Schistosoma mansoni* and *Fasciola gigantica* in Egypt respectively. The larvae spoiled and consumed considerable numbers of egg masses and consequently reduced the rate of hatching. Large larvae (4th instar) destruct also newly hatched *B. glabrata* snails (< 3mm). The consumption of *Lymnaea* egg masses by larvae was lower than in the case of *B. glabrata* probably due to the thicker gelatinous material surrounding the eggs. *P. alternata* larvae were found also to consume *S. mansoni* miracidia and cercariae and their presence during miracidial exposure of snails reduced markedly the infection rate of *B. glabrata* snails with *Schistosoma* and considerably decreased the total periodic cercarial production of infected snails. Therefore *P. alternata* larvae may play an important role in reducing the population density of medically important snails, free- living larval stages of *S. mansoni* and the schistosome infection of target snail with this parasite. Consequently *P. alternata* may be useful in the control of schistosomiasis transmission in aquatic habitats.

Key words: *Psychoda alternata, Biomphalaria glabrata*, *Lymnaea natalensis*, biological control, Egypt.

INTRODUCTION

Schistosomiasis is a serious public health problem in Egypt and many other tropical and subtropical countries, being second most important parasitic disease. Fascioliasis is a well known veterinary problem which is to date an emerging human disease, the WHO has estimated that 2.4 million people allover the world are infected with *Fasciola.* The fight against the host snails is important in any integrated control program which includes also chemotherapy and health education. Methods of snail control comprise the use of chemical molluscicides though their application could give only a temporary relief. Besides having possible adverse effect on the flora and fauna, molluscicides are toxic and expensive (Aditya and Raut, 2002). On the other hand, the biological control comprises the use of living organisms, whether introduced or otherwise manipulated, which can reduce the population density of the target snail species and the free living stages of the parasite. These organisms may be categorized as predators, parasites, parasitoids, pathogens and competitors.

Predators of snails may be polyphagous or very specialized in their prey choice. Example of the known malacophagous or destructive insect predators of snails is larvae of sciomyzid flies (Diptera: Sciomyzidae) (Maharaj *et al.*, 1992*,* 2005; Appleton *et al.,* 1993*;* Rory *et al*., 2007). The objective of this study is to evaluate the potentiality of larvae of another aquatic fly, namely *Psychoda alternate* Say (Diptera: Psychodidae), as a predator against certain medically important snails in Egypt, *Biomphalaria glabrata* and *Lymnaea natalensis,*the snail hosts of *Schistosoma mansoni* and *Fasciola gigantica* respectively, and against the free- living stages of *Schistosoma mansoni*.

Psychoda alternata **(**Drain fly) (Fig 1) belongs to family Psychodidae, suborder Nematocera, order Diptera. It gets its common name from its tendency to live and reproduce in shallow and polluted water. The adult flies are dark grey, triangular in shape, 3- 5 mm in length. It is characterized by dense covering of long hair on the body and wings. The adult has long antennae (12-15 segments) and the wings are leaf-shaped. Psychodidae

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are mostly nocturnal and feeds on organic matter and micro organisms. They are unharmful and cannot sting. The female lays eggs (20- 100) in irregular shape on the surface of plants and sides of drains. The eggs hatch in 48 h into small pale worm like larvae (1-2mm) which molt seven times to reach maturity in 9-15 days. The mature larvae are sub-cylindrical with narrow head. Pupal period is 20-40 h and the whole life cycle is about 21-27 days. There are several generations a year (Vaillant, 1971; Quate and Brown, 2004).

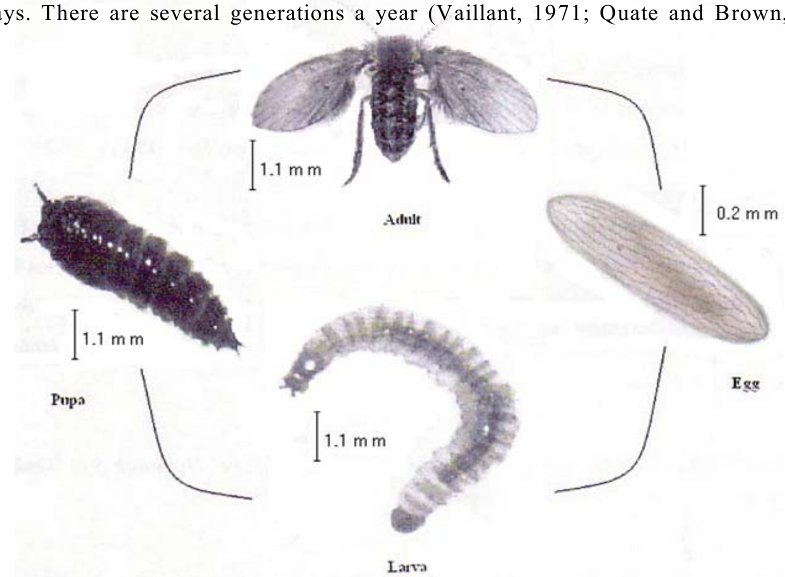

Fig. 1: Life cycle of *Psychoda alternate* Say (Photos by the authors)

MATERIAL AND METHODS

A limited life cycle of *Psychoda* was established in the laboratory so that sufficient numbers of larvae were produced for the experiments. Adult flies were caught by sweeping the vegetation along the banks of Mansouriya Drain, Giza Governorate. They were placed in plastic jars containing wet filter paper, carried to the laboratory where they were maintained in covered aquaria to be reared at 28 ºC ±1ºC in an air conditioned room. Newly hatched larvae were obtained from the aquaria and fed on snail tissues. Two snails namely *Biomphalaria glabrata* Say (Planorbidae) and *Lymnaea natalensis* Krauss *(*Lymnaeidae) were used in this study. They were obtained from laboratory stocks maintained in the Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI). The snail egg masses were obtained also from the laboratory colonies in the first day of oviposition.

The predatory or destructive activity of *P. alternata* larvae were investigated on the snails and the egg masses. Three Petri dishes, containing 50 ml dechlorinated water and 5, 10, and 15 egg masses of *B. glabrata,* were provided each with 5 larvae (third instar). These combinations were maintained in the laboratory examined daily using a stereomicroscope and the number of predated or injured snails or egg masses were recorded. Three other Petri dishes were similarly set up as above for replication and another group of Petri dishes without larvae as control. This experiment was similarly carried out using third and fourth instars of larvae. In all cases number of larvae was maintained constant by replacing those passing to pupal stage during the exposure period. Three sizes of snails (baby <3mm, juvenile 5 mm and mature 7 mm) were used.

To elucidate the effect of availability of plant or animal diet in the habitat on the predatory activity of *Psycoda* larvae, three Petri dishes were similarly used as above, one containing blue green algae (*Nostoc muscorum*), another Petri dish contains boiled lettuce leaves, and the third fresh snail tissues. Five egg masses and 5 larvae were introduced to each Petri dish and two replicates were used in each case. The egg masses were examined daily for egg destruction.

The effect of *Psychoda* larvae on the survival of the free- living larval stages of *S. mansoni* (miracidia and cercariae) as well as on infection of *B. glabrata* snails with this parasite was also studied. Thus freshly produced *S. mansoni* miracidia and cercariae were obtained from SBSC. One hundered of miracidia and cercariae were separator placed with 10 4^{th} instar larvae, in different volumes of dechlorinated water (50, 100, 500 and 1000 ml). Two replicates were performed in each case. The survived miracidia and cercariae were recorded in each case after 15, 30 and 60 min using a stereomicroscope.

The potential effect of *Psychoda* larvae on infection of *B. glabrata* snails with *S. mansoni* miracidia was also studied by exposing the snails to miracidia in presence and absence of the larvae. Snails used were of the same size (4 – 5 mm) and divided randomly into two groups 20 each, and each group was exposed in mass to 8-10 miracidia / snail. Freshly hatched miracidia were used within one hour of hatching. The two groups were maintained over night under the same laboratory conditions. Starting from 30days post miracidial exposure and the surviving snails were examined individually for cercarial shedding. This was carried out twice weekly by exposing snails for one hour to 40 watt florescent lamp, 40 cm far, at 26 ºC ± 1ºC. Snails that proved to be positive were isolated and kept in special aquaria in complete darkness. The emerged cercarial suspension in each case was poured in a graduated Petri dish and a few drops of Bouin fluid were added. All cercariae were counted by using a dissecting microscope. The total periodic cercarial production/ snail were determined. T test & chi² was used for analyzing the data.

RESULTS AND DISCUSSION

Predatory Activity of Psychoda Larvae on Biomphalaria glabrata Egg Masses:

The results given in (Table 1) show that in the Petri dishes containing 5 third instar larvae and 5 egg masses per dish, the daily consumption rate during the first 3 days was high (3.3 eggs / larva) and decreased gradually to reach 1.3 eggs / larva in days 6&7. In Petri dishes containing double and triple the numbers of egg masses (i.e. 10 & 15 egg masses per dish), the daily consumption rate was low at the first three days and increased in days 4&5 then decreased after that. In all cases the remaining eggs hatch to baby snails. It is also observed that the consumed eggs/ larva/ day decreased with the increase of available egg masses in the first 3 days but increased with the increase of available egg masses in the following days.

Table 1: Predatory activity of *Psychoda alternata* 3rd instar larvae* on *Biomphalaria glabrata* eggs

Observation day	Parameter	Number of egg masses (eggs)		
		10 (201)	20 (278)	30 (386)
3	Consumed / remained eggs	99 / 102	40 / 238	19 / 367
	% of consumed eggs	49.3	14.3	4.9
	Consumed eggs/larva/day	3.3 ± 0.3	1.4 ± 0.1	0.7 ± 0.1
5	Consumed / remained eggs	49 / 53	82 / 156	109 / 258
	% of consumed eggs	48	34.5	29.7
	Consumed eggs/larva/day	2.5 ± 0.1	4.1 ± 1.5	5.5 ± 1.2
7	Consumed / remained eggs	25 / 28	56 / 100	77 / 181
	% of consumed eggs	47.2	35.9	29.8
	Consumed eggs/larva/day	1.3 ± 0.4	2.8 ± 0.1	3.9 ± 2.5
9	Consumed / remained eggs			27 / 154
	% of consumed eggs			14.9
	Consumed eggs/larva/day			1.4 ± 1.3
	No. hatched eggs (%)	28 (13.9)	100 (36)	154 (39.9)

* Ten larvae, 2 replicates

Using fourth instar larvae, the results (Table 2) show higher predatory activity rate leading to total consumption of eggs in shorter periods. It is observed the number of predated eggs / larva at the first day was almost similar regardless to available number eggs. The consumed eggs decreased in the third day and were mostly consumed in the fifth day of observation.

Table 2: Predatory activity of *Psychoda alternata* 4th instar larvae* on *Biomphalaria glabrata* eggs

Observation day	Parameter	Number of egg masses (eggs)			
		10 (271)	20 (406)	30 (543)	40 (746)
1	Consumed / remained eggs	254 / 17	265 / 141	214 / 329	226 /520
	% of consumed eggs	93.7	65.3	30.4	30.3
	Consumed eggs/larva/day	25.4 ± 10.5	26.5 ± 4.9	21.4 ± 3.7	11.3 ± 1.7
3	Consumed / remained eggs	17 / 0	141 / 0	268 / 61	470 / 50
	% of consumed eggs	100	100	81.5	90.3
	Consumed eggs/larva/day	1.7 ± 0.4	7.1 ± 0.8	12.9 ± 0.2	23.5 ± 1.0
5	Consumed / remained eggs			61 / 0	20 / 30
	% of consumed eggs			100	40
	Consumed eggs/larva/day			3.1 ± 0.8	1.3 ± 0.4
	No. hatched eggs (%)				30 (4)

*Ten larvae, 2 replicates

Predatory Activity of Psychoda Larvae on Lymnaea Egg Masses:

The results (Table 3) show that *Psychoda* larvae consume also *Lymnaea* eggs but in a rate much less than in the case of *Biomphalaria*.The larvae start feeding on the relatively much thicker jelly material of *Lymnaea* egg masses and at day 8 almost half the eggs were consumed.

Table 3: Predatory activity of *Psychoda alternata* 4th instar larvae on* *Lymnaea natalensis* eggs

Observation day	Parameter	Number of egg masses (eggs)		
		10 (340)	20 (908)	30 (1070)
2	Consumed / remained eggs	47 / 293	86 / 822	85 / 985
	% of consumed eggs	13.8	9.5	7.9
	Consumed eggs/larva/day	2.8 ± 1.1	4.3 ± 1.4	4.3 ± 0.6
4	Consumed / remained eggs	45 / 248	122 / 700	105 / 880
	% of consumed eggs	15.4	14.8	10.6
	Consumed eggs/larva/day	2.3 ± 0.2	6.1 ± 1.9	5.3 ± 2.8
6	Consumed / remained eggs	47 / 201	144 / 556	121 / 759
	% of consumed eggs	18.9	20.6	13.8
	Consumed eggs/larva/day	2.4 ± 1.3	7.2 ± 1.3	6.1 ± 0.4
8	Consumed / remained eggs	44 / 157	163 / 393	191 / 568
	% of consumed eggs	21.9	29.3	25.2
	Consumed eggs/larva/day	2.2 ± 0.7	8.2 ± 2.2	9.6 ± 1.9
	No. hatched eggs (%)	157 (46.2)	393 (43.3)	568 (53.1)

* Ten larvae, 2 replicates

Predatory Activity of Psychoda Larvae on Biomphalaria and Lymnaea Snails:

The results show that *B. glabrata* snails (< 3 mm in shell diameter) were injured by 4th instar *Psychoda* larvae, about 2 thirds of the snails were distructed during the observation period (7 days). Meanwhile almost no effect was observed on the newly hatched lymnaeid snails as well as juvenile snails of both spp. (*Biomphalaria* and *Lymnaea).*

Predatory Activity of Psychoda Larvae on Snails in Presence of Other Food Diet:

In the first day, the larvae did not harm the snail eggs in the presence of the tested types of diet namely algae, boiled lettuce and snails tissues. In the following days the results (Table 4) show that the availability of other diet reduces the consumption of snail eggs. This reducing effect is less in the presence of snail tissues rather than with the algae and lettuce leaves.

Table 4: Predatory activity of *Psychoda alternata* 4th instar larvae* on *Biomphalaria glabrata* eggs in presence of other diet.

Observation day	Parameter	Type of food with of *B. glabrata eggs*			
	Food type + (eggs)	Algae + (191)	Lettuce + (184)	Snail tissues + (215)	Control (271)
3	Consumed / remained eggs	57 / 134	51 / 133	78 / 137	244 / 27
	% consumed eggs	29.8	27.7	36.3	90
	Consumed eggs/larva/day	1.9 ± 1.4	1.7 ± 1.5	2.6 ± 1.2	8.1 ± 10.05
7	Consumed / remained eggs	18 / 116	41 / 92	99 / 38	27 / 0
	% consumed eggs	13.4	30.8	72.3	100
	Consumed eggs/larva/day	0.6 ± 1.7	1.3 ± 1.6	3.3 ± 8.9	1.9 ± 0.4
9	Consumed / remained eggs	45 / 71	29 / 63	38 / 0	
	% consumed eggs	38.8	31.5	100	
	Consumed eggs/larva/day	2.3 ± 1.1	1.5	1.9 ± 1.4	
	No. hatched eggs (%)	71 (37.2)	63 (43.2)	0	0

* Ten larvae, 2 replicates

Predatory Activity of Psychoda Larvae on Free – Living Stages of Schistosoma mansoni (miracidia& cercariae):

Fig (2) shows that the predated miracidia or cercariae by larvae increased significantly ($p<0.01$) with time (15–60 min). But, when the volume of water increased the consumption of these organisms significantly decreased ($p<0.01$) and was minimal in the case of 1000 ml water. It is observed also that the number of predated miracidia by the larvae was much less than in case of cercariae ($p<0.01$). This indicates that the capability of the larvae to finding cercariae is more than that in the case of miracidia.

The larvae consumption of miracidia and cercariae increased with the increase of organism density, ($p<0.001$) reaching 14.6& 28 / larva / hour in the case of miracidia and cercariae, respectively (Fig 3).

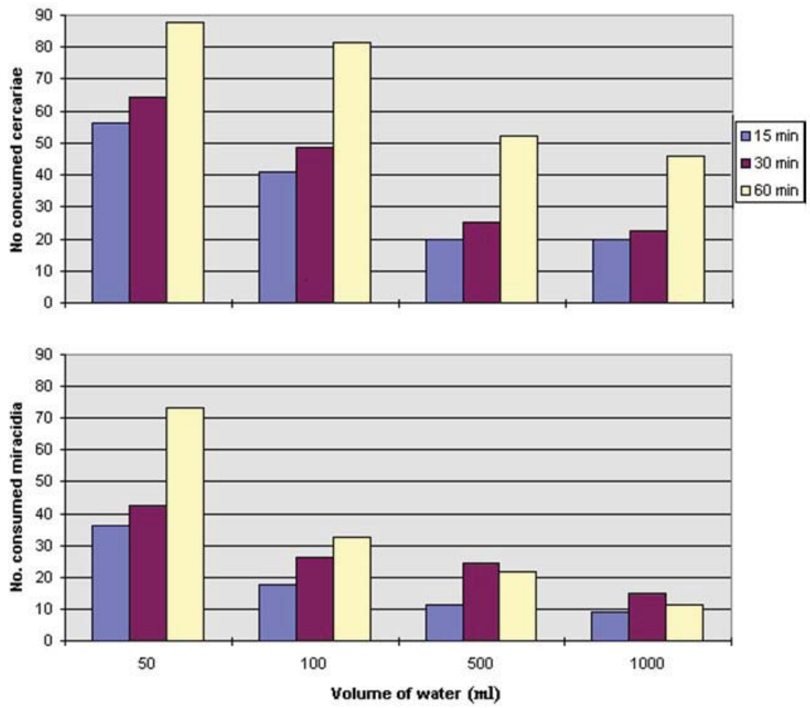

Fig. 2: Predatory activity of *Psychoda alterata* (4th instar) larvae on *Schistosoma manson* free larval stages (100 organisms) using various volumes of water after various observation periods (15, 30, 60 min).

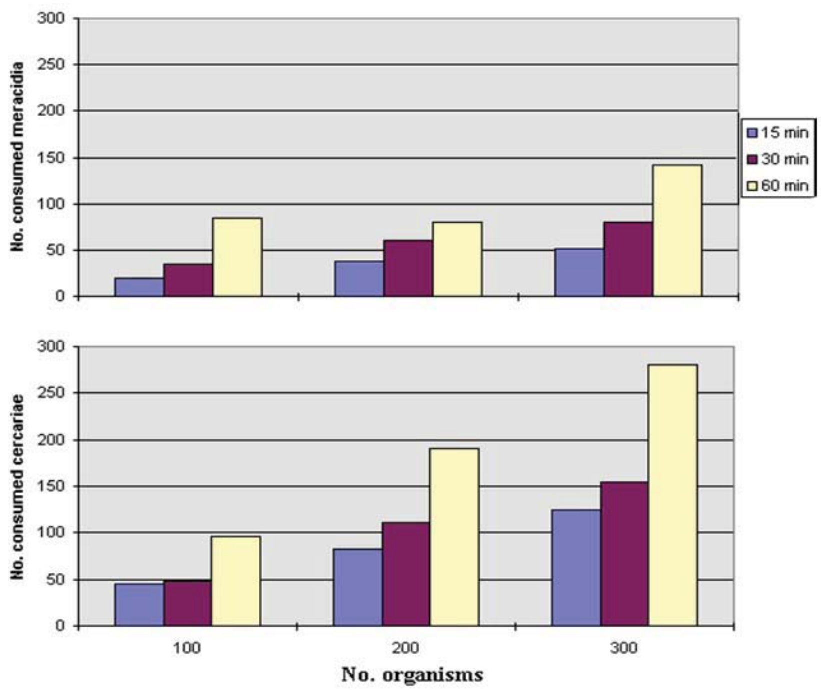

Fig. 3: Predatory activity of *Psychoda alternate* 4th instar larvae on various densities of *Schistosoma mansoni* free larval stages after observation periods (15, 30, 60 min).

Effect of Psychoda Larvae on Infection of B. glabrata with S. mansoni Miracidia:

The infection rate of *B. glabrata* with *S. mansoni* was significantly reduced in snails which were associated with *Psychoda* fourth instar larvae during miracidial exposure than the control group (p> 0.001). However, the prepatent period showed no difference between the two groups while the total periodic cercarial production/ snail decreased significantly (p <0.001) (Table 5).

Table 5: Effect of *Psychoda alternata* larvae (4th instar) on the infection of *Biomphalaria glabrata* with *Schistosoma mansoni* miracidia

Parameter	Control	Experimental	% Change
Snail infestation (%)	90	20	-77.8
Prepatent period (day)	35.6 ± 4.2	35.6 ± 4.2	0
Total periodic cercarial production / snail	6792.7 ± 2615	3523.1 ± 2547.4	-62.5

Discussion:

The present study under laboratory conditions demonstrates the predatory activity of *Psychoda* larvae against *B. glabrata* snails (<3 mm) and the snail eggs, as well as on the survival of the free- living larval stages of *S. mansoni* these larvae caused also reduction on infection of the vector snails with schistosome miracidia. Throughout the duration of the experiment (<9days) several variables occur. Growth, molting and pupation of the larvae and growth and development of the snail embryos within the eggs these affect considerably the rate of consumption of the larvae. The larvae feed at first on the gelatinous material surrounding the egg masses and therefore consume fewer numbers of embryos in the first few days. This consumption is significant in the case of lymnaeid than planorbid eggs because the former egg masses have much more thick gelatinous material surrounding the eggs.

The present results show that larger larvae of *P. alternata* have more predatory activity than younger ones; thus the fourth instar larvae are more voracious than the third one. In this respect, the present findings agree with that found by Manguim and Vala (1989) who claimed that the number of preys consumed by *Tetanocera ferruginea* (Diptera: Sciomyzidae) increased as larvae grew. Similar effect was found by Maharaj *et al.,* (1992) and Foote and Keiper (2004) who found that older sciomyzid larvae can feed more as larvae grow. Such changing pattern may be an indication of the increasing or changing nutritional demands of each stage. Meanwhile, the number of snail eggs eaten by the larvae was found to decrease with the development of the snail embryos and or with the coming of the larvae to pupation, a feature reported also by Maharaj *et al.*, (2005). In the present work, the fourth instar *P. alternata* larvae can only kill *Biomphalaria* snails < 3mm but not *Lymnaea* snails of the same size. Raut and Saha (1989) who claimed that snail predation by larvae is influenced by the morphology and behavior of the mollusc which make it more or less vulnerable. Such larval feeding preference was recently indicated in the case of the sciomyzid fly *Sepedon scapularis* by Maharaj *et al.* (2005).

The present results show that *Psychoda* larvae are not obligatory malacophagous and can consume vegetation. However this may not effect their activity against snail eggs which are usually laid on the lower surface of the aquatic plant leaves.

The present results show also that the free living schistosome larval stages (miracidia & cercariae) were consumed by *Psychoda* larvae. The predation on these organisms decreased (p<0.001) with the increase of water volume and increased with the increase of their density in all exposure periods. Similar findings were given by Muraheedharan *et al.,* (1975) and by Gawish *et al.,* (2006).

The present results show also that experimental infection of *B. glabrata* snails with schistosome miracidia was significantly lower than control group (p > 0.001) and the total periodic cercarial production was significantly decreased (p <0.001). Similar effect reported in some other cases such as with *Daphnia pulex* (Cladocera) which significantly protected *L. truncatula* snails from infection with *F. hepatica* (Christensen *et al.,* 1977) and in the case of *D. magna* by Gawish *et al.,* (2006).

The present results give indication about the potential effect of *P. alternata* larvae as a biocontrol agent for schistosome transmission these larvae have reducing effect on snail population as well as infection with schistosome in natural habitats. More studies should be performed for correlating natural prevalence of *Psychoda* fly and schistosome transmission in the same sites, as well as conducting pilot trials under controlled simulated natural conditions to verify this hypothesis.

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