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Blepharisma intermedium Padmavathi, 1959 (Ciliophora: Heterotrichida) from Al-Hassa Inland Hypersaline Oasis in Saudi Arabia

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Summary. A medium-sized, pink heterotrich ciliate was found in hypersaline ponds in the inland Al-Hassa Oasis. The morphology and infraciliature were studied *in vivo*, and in silver carbonate and protargol impregnated cells. The organism has a slender filiform macronucleus without terminal swellings. The morphology and morphometric data agree largely with the original description of *Blepharisma intermedium* Padmavathi, 1959; however, the present organism has fewer kineties and both kinetosomes of somatic dikinetids are ciliated. The findings are discussed on the basis of a summary made from available data on other *Blepharisma* species with a filiform macronucleus.

Key words: *Blepharisma intermedium*, hypersaline ponds, infraciliature, inland oasis, protargol impregnation.

INTRODUCTION

The Al-Hassa Oasis is one of the largest in the world. It is situated some 50 km inland from the Arabian Gulf coast, West of the vast sand desert of Al Jafurah (25° 30' N; 49° 40' E). This oasis is an inland sabkhah with some similarities to the coastal sabkhas of the Arabian Gulf (Johnson *et al.* 1978). There are numerous wells and artesian springs with abundant fresh water, which makes the oasis an important producer of dates and other agricultural products. The ground water level of the oasis is particularly shallow, which causes mixing of fresh water with the salty sediments, forming scattered ponds of various salinity levels (4-18 ‰). The raised salinity of the ponds may be due to dissolution of salts from soil surfaces (AL-Rasheid 1997). Salinity of the ponds decreases southwards, where the number of springy wells is increases, along with increasing vegetation. Due to high temperature and lack of rain during the long summer season (the mean water temperature is 15° C during January, the coldest month, and 35° C during July, the hottest month of the year), several of the shallow fresh and brackish water ponds turn saline and/or hypersaline (70-160 ‰). These ponds are surrounded by the salt-tolerant tallreed (*Phragmites*) and mangroves (*Avicennia*). The ciliate fauna of the oasis has been studied by AL-Rasheid (1997) who reported

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37 species of typical marine ciliates including the two well known saline-tolerant species, *Fabrea salina* and *Condylostoma reichi*.

A medium-sized, pink *Blepharisma* sp. with filiform macronuclei has been found in hypersaline ponds in the Saudi Arabian inland oasis Al-Hassa. Members of the genus *Blepharisma* are widespread and have been reported from fresh, brackish and sea water as well as from soils of many parts of the world (see for example Borror 1963; Isquith *et al.* 1965; Kattar 1965; Nilsson 1967; Dragesco 1970; Larsen 1982, 1983, 1992; Larsen and Nilsson 1983, 1988; Dragesco and Dragesco-Kernéis 1986, 1991; Foissner 1989; Foissner and O'Donoghue 1990; Aescht and Foissner 1998; AL-Rasheid 1999). A few species have been found in extreme hypersaline habitats, such as *B. halophila* (Ruinen 1938, Post *et al.* 1983), *B. dileptus* and *B. tardum* (Kahl 1928) and *Blepharisma* sp. (Wilbert 1995). The taxonomy of the genus *Blepharisma* has undergone several taxonomical revisions starting with Kahl (1932) then Bhandary (1962), Hirshfield *et al.* (1965, 1973) and finally Repak *et al.* (1977).

The aim of the present work is to describe a population believed to be of *Blepharisma intermedium* found in Saudi Arabia and to give some information regarding the taxonomy of blepharismas with filiform macronuclei.

MATERIALS AND METHODS

The organism was found in hypersaline ponds (100-160 ‰ salinity) North of the Oasis on several occasions during the summer and winter seasons of 1999 and 2000. Freshly collected sediment and water samples were studied on site. Several specimens of the ciliate organisms were collected, studied *in vivo* then in protargol and silver carbonate-stained preparations (Foissner 1991). Several short and long-term cultivation methods were conducted, but with no success as the cultures declined and died out rapidly. Specimen preparations of protargol and silver carbonate impregnated cells have been deposited in the Museum of Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia.

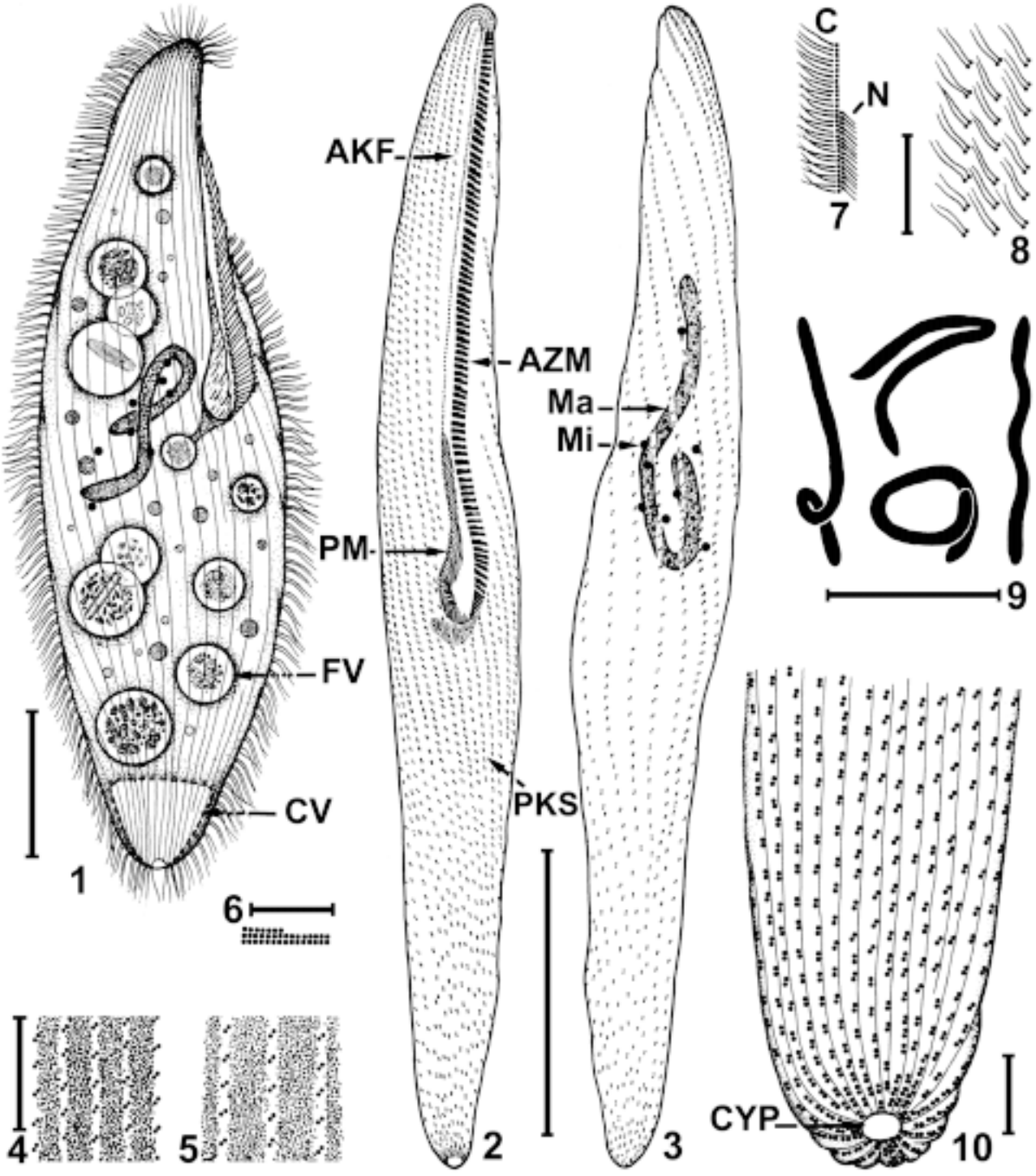
OBSERVATIONS

The Saudi Arabian organism is slender, rounded anteriorly and posteriorly, and has a size of 239-359 x 39-52 µm (*in vivo ca* 250 - 400 x 44 - 60 µm) (Figs. 1, 11; Table 1). The pellicle is flexible with numerous dark pink subpellicular granules (0.4-0.5 µm in diameter) arranged in rows of approximately 8-12 granules across between the ciliary rows of kineties (Figs. 4, 5, 13, 14). Food vacuoles may be abundant, filled with bacteria, algae and diatoms (Figs. 11, 12). The contractile vacuole is found terminally (Fig. 1). The oral groove is long and narrow starting slightly below the anterior pole and stretching posteriorly to about the middle of the body (Figs. 2, 17). The entire left side of the groove is furnished with an adoral zone of membranelles (Fig. 15) in which each membranelle base consists of rows of basal bodies, two long and one short (Fig. 6). The right side is occupied by a paroral membrane, which ends posteriorly at the cytostome. The anterior third of the paroral membrane consists of a line of closely spaced basal bodies, while the remaining part consists of zigzagging dikinetids having only the right basal body ciliated (Fig. 15). Single long fibers (nematodesmata) originate from the left basal bodies in the posterior third of the paroral membrane and extend over the buccal cavity and enter the cytopharynx as oral ribs (Fig. 7). There are 25 (21-27) somatic kineties; on the right side of the organism the rows of cilia run parallel to the oral groove, while on the left side the rows run obliquely (Figs. 2, 3, 13, 14; Table 1). The kineties consist of paired basal bodies and both are ciliated (Fig. 8). The somatic cilia are about 6-9 µm long. The kineties stop short just before the posterior end leaving a bare cytopyge free of cilia and subpellicular granules (Fig. 10). The single filiform macronucleus is without terminal swellings (Figs. 1, 3) and occurs in many twisted configurations (Fig. 9). The 5-9 spherical micronuclei, each about 2 µm in diameter, are located close to the macronucleus (Figs. 3, 16; Table 1).

Cultivation was unsuccessful, so it was not possible to study cell division, yet a few early and late postdividers were found naturally in several samples collected on different occasions. Examples are recorded in Figs. 18, 19 for future studies.

DISCUSSION

In order to identify the present Saudi Arabian blepharisma taxonomically, a summary (Table 2) was made of the available data from the literature on *Blepharisma* spp. with filiform macronuclei. The present Arabian organism matches in some aspects *Blepharisma intermedium* Padmavathi (1959), e.g. in the general size of the organism, in shape of the filiform macronucleus, and in some other morphometric features (see Table 2). However, the number of somatic kineties is lower (21-27) in the Arabian blepharisma than the 42-64



Figs. 1-10. *Blepharisma intermedium* from life (**1**), after protargol impregnation (**2, 3, 6-10**) and after silver carbonate impregnation (**4, 5**). **1** - right lateral view of a compressed organism showing the general appearance, many food vacuoles and the terminal contractile vacuole; **2, 3** - infraciliature of right and left sides showing adoral zone of membranelles, paroral membrane, macronucleus and micronuclei; **4, 5** - details of pellicle with subpellicular cortical granules of right and left sides; **6** - detail of kinetosome arranged in adoral membranelle; **7** - detail of paroral membrane; **8** - detail of somatic infraciliature showing that both kinetosomes in a pair are ciliated; **9** - macronucleus variations; **10** - somatic infraciliature of the posterior end showing that the cytopyge opening is free of kinetosomes. AKF - anterior kinety fragment, AZM - adoral zone of membranelle, C - cilia, CV - contractile vacuole, CYP - cytopyge, FV - food vacuole, Ma - macronucleus, Mi - micronucleus, N - nematodesma, PM - paroral membrane, PKS - shortened postoral kinety. Scale bars - 75 µm (Figs. 1-3, 9), 15 µm (Figs. 4, 5,7, 8, 10) and 5 µm (Fig. 6)

Table 1. Morphometric characteristics of *Blepharisma intermedium* (Data based on randomly selected protargol-impregnated specimens). Measurements are in µm

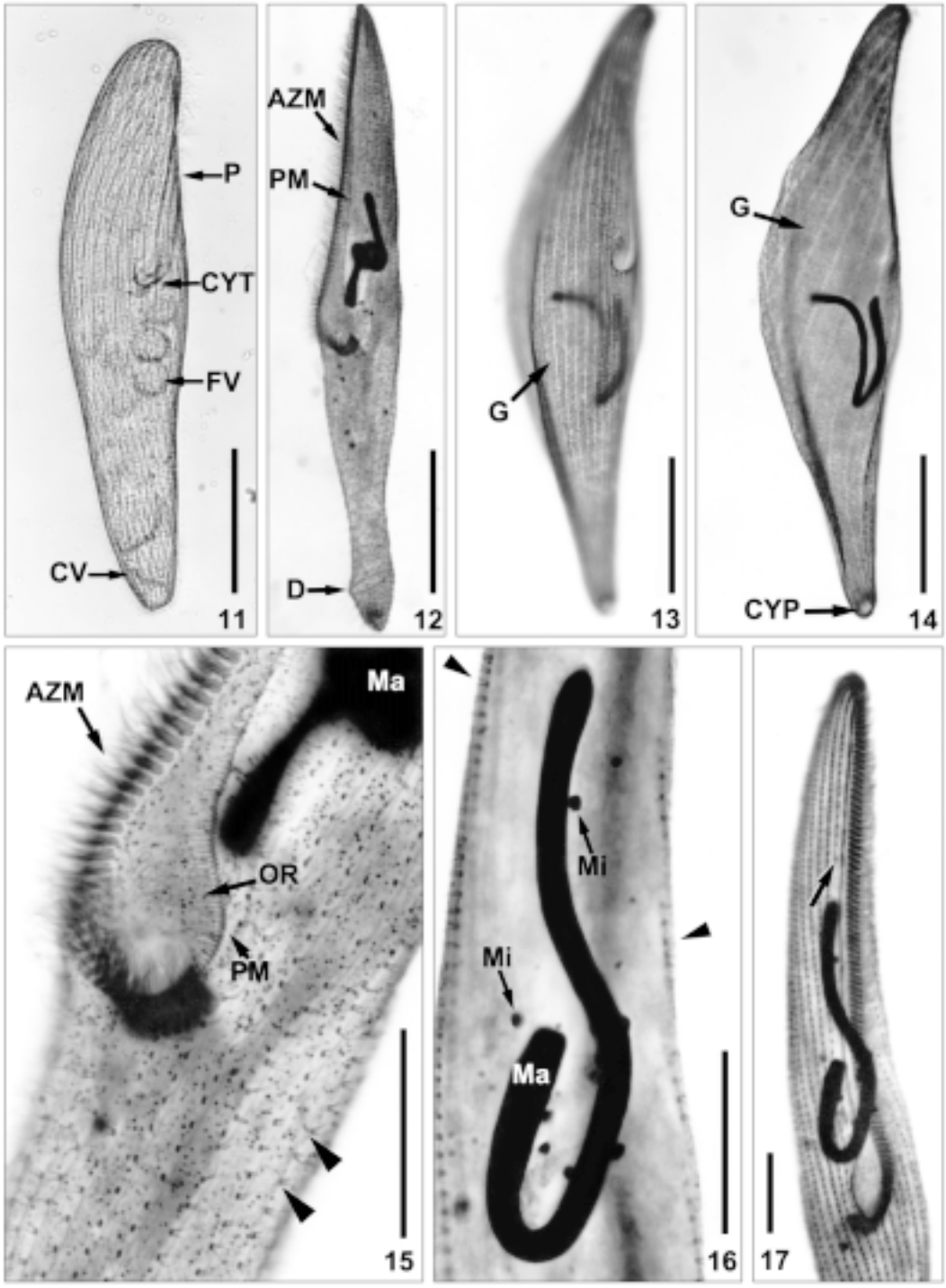
Character	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	306.3	314	43.1	3.6	14.1	239	359	10
Body, maximum width	44.8	45	4.3	1.4	9.6	39	52	10
Adoral zone of membranelles, length	144	151	16.8	5.3	11.7	119	171	10
Adoral zone of membranelles, width	5.0	5.1	0.2	0.1	4.0	4.5	5.2	20
Adoral membranelles, number	86.4	87.5	6.8	2.2	7.9	76	98	10
Paroral membrane, length	71.2	73.5	10.9	3.4	15.5	54	83	10
Macronucleus, length	131	130	12.9	4.1	9.8	114	156	10
Macronucleus, diameter	3.8	3.9	0.2	0.1	5.3	3.2	4.1	30
Micronuclei, diameter	2.3	2.3	0.3	0.1	13.0	1.8	2.7	30
Micronuclei, number	7.1	7.0	1.3	0.2	18.3	5	9	10
Somatic kineties, number postoral	25	26	1.8	0.6	7.2	21	27	10

CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of individuals investigated, SD - standard deviation, SE - standard error of the mean, $\bar{x}$ - arithmetic mean

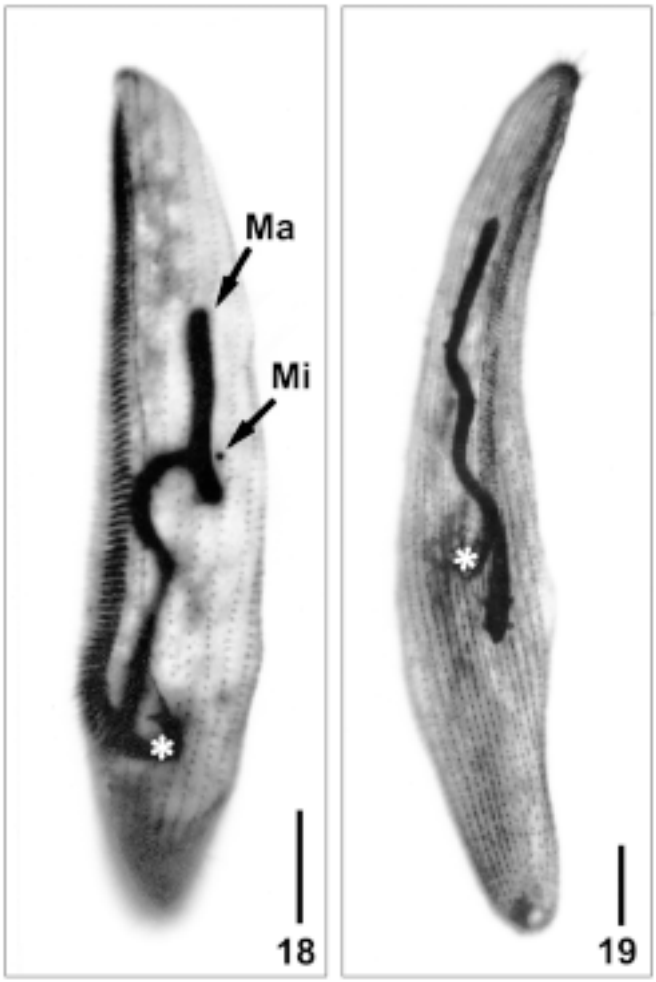
Table 2. Comparison of some reported characteristics of *Blepharisma* with filiform macronucleus

Species Author	*B. japonicum* Suzuki 1954	*B. japonicum* Nilsson 1967	*B. japonicum* Dragesco 1970	*B. japonicum* Dragesco & Dragesco-Kernéis 1986	*B. stoltei* Isquith 1966**	*B. briviliformis* Isquith 1966**	*B. intermedium* Padmavathi 1959	*B. intermedium* present study
Locality	Japan	Uganda	Cameroon	Uganda	Japan	Japan	India	Saudi Arabia
Length µm	150-500	250-700	400-650	225-400	145-225	120-195	200-350	239-359
Width µm	62-215	90-260*	100-200	80-150	45-85	35-90	45-100	39-52
Peristome length µm	75-250	75-350	150-230	100-185	70-125	50-85	70-140	119-171
Somatic kineties number	50-70	60-100	54	76	50	36-54	42-64	21-27
AZM number	91	90-178*	80	112	72	42-57	82	76-98
PM length µm	30-50	50-230	50-110	25-50	24-60	15-35	25-50	54-83
PM type	prominent	prominent	prominent	prominent	not prominent	not prominent	not prominent	not prominent
Terminal swelling of macronucleus	conspicuous	conspicuous	inconspicuous	conspicuous	conspicuous	inconspicuous	inconspicuous	inconspicuous
Micronuclei diameter	1.2	1	1.8-2.8	2	1.5-2	?	1.97	1.8-2.7
Micronuclei number	2-22	15-25	13-26	15-25	4-18	2-7	6-30	5-9
Subpellicular granules color	deep red	deep red	red	reddish	pale pink	?	dark pink	dark pink
Subpellicular granules number	6-10	5-8	?	?	3-6	?	?	8-12

* unpublished data, ** see Hirshfield *et al.* (1973)



Figs. 11-17. Light micrographs of *Blepharisma intermedium* from life (**11**), after protargol impregnation (**12, 15-17**) and after silver carbonate impregnation (**13, 14**). **11** - right lateral view of a slightly compressed organism showing the general appearance; **12** - left lateral view showing the macronucleus, somatic kineties and a food vacuole containing a diatom (D); **13, 14** - right lateral views at different focus showing the arrangements of the longitudinal and oblique subpellicular granules of the right and left sides (the cells are swollen due to the silver carbonate technique); **15** - part of Fig. 12 at higher magnification showing the adoral zone of membranelles, macronucleus, the zigzagging part of the paroral membrane and the oral ribs. Arrowheads indicating that both paired kinetosomes of the dikinetids are ciliated; **16** - the filiform macronucleus with inconspicuous terminal swelling and the micronuclei. Arrowheads indicating that both paired kinetosomes of the dikinetids are ciliated; **17** - ventral view showing the anterior kinety fragment and other parts of the peristome. AZM - adoral zone of membranelles, CV - contractile vacuole, CYP - cytopyge, CYT - cytostome, D - diatom, FV - food vacuole, G - subpellicular granules, Ma - macronucleus, Mi - micronucleus, OR - oral ribs, P - peristome, PM - paroral membrane. Scale bars - 75 µm (Figs. 11 - 14), 25 µm (Figs. 15, 16) and 50 µm (Fig. 17)



Figs. **18-19**. Light micrographs of *Blepharisma intermedium* after protargol impregnation. Early (**18**) and late (**19**) postdividers. Cytostome positions are marked by asterisks. Ma - macronucleus, Mi - micronucleus. Scale bars - 25 µm

kineties reported originally by Padmavathi (1959) for *B. intermedium*. In revisions of the genus *Blepharisma* (Bhandary 1962; Hirshfield *et al.* 1965, 1973), the number of somatic kineties in *B. intermedium* is not mentioned. An unusual low number of 25-35 kineties was also found by Sawyer (1977) in *B. japonicum* Suzuki, so this feature may be unimportant (see Table 2).

A striking feature of the Arabian ciliate is, however, that both kinetosomes of the somatic pairs are ciliated. This is an unusual feature in *Blepharisma* where most descriptions on the different species state that only one kinetosome of the somatic pairs is ciliated. To the best of our knowledge, the only other blepharisma reported to have both kinetosomes of somatic pairs ciliated, is *B. parasalinarium* (Dragesco 1996). This organism does not have a filiform macronucleus but about 60 small irregular, spheroid macronuclei which rules out any close relationship to the present Arabian organism.

The colour of the ciliate is not an accurate taxonomical feature, as it is well known that the *Blepharisma* pigment bleaches on exposure to light. The dark pink colour of the present organism may be explained by the heavy vegetation shading the saline ponds where it was found.

In spite the fact that the somatic pairs of kinetosomes are both ciliated, an unusual feature, we conclude that the present organism with its filiform macronucleus is an Arabian strain of the freshwater *Blepharisma intermedium* Padmavathi (1959).

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