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Body, Nuclear, and Ciliary Changes during Conjugation of *Protospathidium serpens* (Ciliophora, Haptoria)

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ABSTRACT. We studied cell size and shape, nuclear changes, and the ciliary pattern during conjugation of *Protospathidium serpens*, using protargol impregnation and morphometry. Preliminary data were gathered from *Epispathidium ascendens* and *Apertospathula armata*. Conjugation of *P. serpens* is temporary, isogamic, and without preconjugation divisions. Pair formation is heteropolar, and the partners unite obliquely with the oral bulge. The body becomes smaller and broader during conjugation, but no basic changes occur in the ciliary pattern. Conjugation and nuclear reconstruction follow the usual mode of ciliates. However, some peculiarities occur: only two of the four synkaryon derivatives of the second synkaryon division enter the third division and generate four macronuclear anlagen, which fuse to a single, long macronucleus strand. During conjugation, *E. ascendens* unites obliquely as *P. serpens*, while *A. armata* can pair dorsal-to-dorsal surface, ventral-to-dorsal surface, or obliquely as *P. serpens*. The nuclear processes of these three species are also rather different, showing a considerable diversity in union modes and nuclear events of spathidiids; *E. ascendens* even has preconjugation division. Confirming previous data, the present study shows convincingly that most of the spathidiid nuclear variability is caused by reconstruction processes occurring in post-dividers, exconjugants and, possibly, exautogamonts. When these specimens are removed from the populations, spathidiid species are as stable (or variable) as other ciliate species.

Key Words. *Apertospathula armata, Epispathidium ascendens*, exconjugant, preconjugant, soil ciliates, *Spathidium*, taxonomy, union modes.

THE spathidiid haptorids are a highly diverse ciliate group, whose species are frequently considered as almost indeterminable because some important features, for instance, body size and nuclear apparatus, appear rather variable. Indeed, unusual specimens with different body size and nuclear pattern occur frequently in preparations from natural populations (Foissner 1981, 1984, 1988; Foissner, Agatha, and Berger 2002; Foissner et al. 2004). Literature data suggest that a good deal of the variability is caused by long-lasting nuclear reconstruction processes in post-dividers (Foissner, Agatha, and Berger 2002) and by endomixis in excysting cells (Moore 1924a, b). Our study shows a third reason, viz., conjugation.

Reliable and detailed data on conjugation of haptorids are very scant (Raikov 1972): descriptions are available from *Didinium* (Prandtl 1906), *Dileptus* (Vinnikova 1974; Visscher 1927), and *Acaryophrya* (Serrano, Martín-González, and Fernández-Galiano 1990). The sole study on *Spathidium* is that of Woodruff and Spencer (1924), who provided pioneer data on body changes, duration of conjugation, and the four macronuclear anlagen in exconjugants. Our study was initiated by the rather high frequency of specimens with unusual body size and nuclear pattern in various *Spathidium* s. l. species. Spontaneous mass conjugation of *Protospathidium serpens* and *Epispathidium ascendens* then provided an excellent possibility to study the process in detail.

MATERIALS AND METHODS

Protospathidium serpens (Kahl, 1930) Foissner, 1981 was found in a soil sample (pH 6.5) from the dry bed of the Mlambane River in the Kruger National Park, Republic of South Africa. Cultures were established in Eau de Volvic (French table water) enriched with some drops of percolate from the nonflooded Petri dish culture (Foissner 1987a) and a few crushed wheat grains to stimulate growth of bacteria and prey protozoa (*Chilomonas* sp., *Cyrtolophosis mucicola*, middle-sized colpodas, and hypotrichs). Spontaneous mass conjugation occurred when the culture was rather old, that is, during the stationary growth phase in August 1992. Additional data were obtained from an Austrian population of *Epispathidium ascendens* (Wenzel, 1955) Foissner, 1987 and a Greek population of *Apertospathula armata* Foissner, Agatha & Berger, 2002. Both occurred in non-flooded Petri dish cultures set up with soil and leaf litter from the upper 10 cm. In *E. ascendens*, spontaneous mass conjugation occurred in the raw culture.

Species were identified according to Foissner (1981, 1987b, 1996) and Foissner, Agatha, and Berger (2002), using live observation and protargol impregnation. When conjugation was recognized, the cultures were fixed and protargol-impregnated with protocol A described in Foissner (1991). The conjugation events were reconstructed from these preparations, which show both, the ciliary pattern and the nuclear apparatus very well. Usually, the various macro- and micronuclear processes could be distinguished by their different affinity to protargol, ranging from yellowish to black. The preparations were deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI), Austria. Relevant specimens were marked by black ink circles on the cover glass.

TERMINOLOGY

Terminology is mainly according to Raikov (1972) and Corliss (1979). As it is complex and some new terms are introduced, we provide concise definitions. *Derivatives*: in a general sense, these are products of micronuclear divisions (Raikov 1972). We distinguish between "*maturation derivatives*" (products of maturation divisions preceding formation of pronuclei) and "*synkaryon derivatives*" (products of synkaryon divisions preceding differentiation of macronuclear anlagen and micronuclei). *Dorsal-to-dorsal* (oral bulge) union mode: the partners unite in a way that the dorsal and ventral ends of the oral bulge point in the same direction; the brush of both partners is visible if specimens are observed in the same focal plane (Fig. 43). *Exconjugant division* (= metagamic cell division): cell division segregating the macronuclear anlagen. *Exconjugant nuclear reconstruction*: nuclear events after separation of conjugants. *Heteropolar* pair formation: the cell ends of the conjugants are oriented in opposite directions (e.g. in *Protospathidium* (Fig. 1) and *Dileptus*). *Homopolar* pair formation: the cell ends of the conjugants are oriented in the same direction (e.g. in *Tetrahymena* and *Paramecium*). *Macronuclear anlage*: product of the last synkaryon division. *Maturation divisions* (= progamic micronuclear divisions): the two meiotic and one equational (postmeiotic) micronuclear divisions preceding formation of pronuclei. *Oblique* (oral bulge) union mode: the partners unite more or less obliquely; usually, the brush of both partners is partially recognizable (Fig. 46). *Preconjugant*: offspring of preconjugation division. *Preconjugation division* (= progamic cell division): special division preceding conjugation.

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Table 1. Morphometric data on vegetative and conjugating *Protospathidium serpens*.

Stage[a]	Characteristics	x̄	M	SD	SE	CV	Min	Max	n
Vegetative	Body, length	68.9	67.0	7.6	1.6	11.0	58.0	83.0	22
	Body, width	13.2	13.5	2.7	0.6	20.4	8.0	19.0	22
	Body length : width, ratio	5.4	5.4	1.0	0.2	18.7	3.7	7.3	22
Early conjugants[b]	Long partner, length	68.5	67.5	6.2	2.5	9.1	60.0	77.0	6
	width	16.3	16.5	2.0	0.8	12.0	13.0	19.0	6
	length : width ratio	4.2	4.2	0.3	0.1	6.3	3.9	4.6	6
	Short partner, length	62.8	61.5	4.4	1.8	6.9	58.0	69.0	6
	width	15.0	15.0	1.4	0.6	9.4	13.0	17.0	6
	length : width, ratio	4.2	4.1	0.6	0.3	14.7	3.5	5.3	6
First maturation division[c]	Long partner, length	49.8	50.0	6.9	2.1	13.8	38.0	61.0	11
	width	18.5	18.0	2.5	0.8	13.7	14.0	22.0	11
	length : width ratio	2.8	2.7	0.7	0.2	24.8	1.7	4.1	11
	Short partner, length	44.0	44.0	5.0	1.5	11.4	35.0	50.0	11
	width	17.1	18.0	2.6	0.8	15.1	12.0	20.0	11
	length : width, ratio	2.7	2.3	0.7	0.2	27.0	1.9	4.2	11
Second and third maturation divisions	Long partner, length	40.4	40.0	4.9	1.5	12.0	35.0	52.0	11
	width	19.2	20.0	1.9	0.6	10.1	16.0	21.0	11
	length : width ratio	2.1	2.1	0.3	0.1	15.6	1.8	2.7	11
	Short partner, length	36.5	37.0	4.3	1.3	11.9	28.0	44.0	11
	width	17.6	19.0	2.7	0.8	15.1	13.0	21.0	11
	length : width, ratio	2.1	2.0	0.3	0.1	13.4	1.9	2.8	11
Pronuclei	Long partner, length	41.6	41.0	6.1	1.8	14.6	34.0	56.0	11
	width	18.0	19.0	2.6	0.8	14.3	14.0	21.0	11
	length : width ratio	2.4	2.4	0.4	0.1	19.1	1.8	2.9	11
	Short partner, length	36.2	37.0	2.0	0.9	8.1	32.0	42.0	11
	width	16.9	16.0	2.1	0.6	12.5	15.0	22.0	11
	length : width, ratio	2.2	2.1	0.3	0.1	14.8	1.7	2.8	11
Synkaryon divisions	Long partner, length	37.9	38.0	6.6	2.0	17.3	28.0	48.0	11
	width	19.2	20.0	3.4	1.0	17.7	12.0	24.0	11
	length : width ratio	2.0	2.0	0.5	0.1	22.6	1.4	3.0	11
	Short partner, length	34.3	35.0	4.5	1.4	13.2	27.0	40.0	11
	width	16.1	16.0	2.8	0.8	17.5	10.0	20.0	11
	length : width, ratio	2.2	1.9	0.5	0.2	22.9	1.6	2.9	11
Late conjugants with four macronuclear anlagen	Long partner, length	34.4	35.0	3.9	1.7	11.4	28.0	38.0	5
	width	19.4	19.0	2.3	1.0	11.9	17.0	23.0	5
	length : width ratio	1.8	1.8	0.2	0.1	9.9	1.6	2.0	5
	Short partner, length	30.4	31.0	2.2	1.0	7.2	27.0	33.0	5
	width	15.8	16.0	1.3	0.6	8.3	14.0	17.0	5
	length : width, ratio	1.9	1.8	0.2	0.1	11.5	1.7	2.2	5
Exconjugates with four macronuclear anlagen	Body, length	58.8	60.0	10.8	3.2	18.3	45.0	77.0	11
	Body, width	17.2	18.0	3.6	1.1	21.0	11.0	22.0	11
	Body length : width, ratio	3.6	3.5	1.0	0.3	28.4	2.2	5.1	11
Exconjugants with 2, 3, 5, 6, or 8 macronuclear anlagen	Body, length	69.6	70.0	11.0	3.3	15.8	54.0	85.0	11
	Body, width	16.8	18.0	3.1	0.9	18.4	10.0	21.0	11
	Body length : width, ratio	4.2	4.3	0.7	0.2	17.5	3.4	5.4	11

[a] Data based on mounted and protargol-impregnated (Foissner's method) specimens from a pure culture. Measurements in μm. CV = coefficient of variation in %, M = median, Max = maximum, Min = minimum, n = number of individuals investigated, SD = standard deviation, SE = standard error of arithmetic mean, x̄ = arithmetic mean.

[b] Early conjugants still have a 'good' macronucleus strand, which, however, commences to condense. The micronuclei are in very early prophase, i.e., are slightly inflated.

[c] During this process the macronucleus is more or less distinctly shortened but not fragmented, as shown in Fig. 3.

Pronuclei (= gamete nuclei, viz., the migratory and the stationary): haploid products of the maturation divisions, fusing during karyogamy. *Reconjugation*: mating of exconjugants that have not yet completed nuclear reconstruction; bilateral if two exconjugants mate, unilateral if an exconjugant mates with an ordinary specimen. *Synkaryon*: diploid product of fusion of the pronuclei during ordinary conjugation (heterozygous) and autogamy (homozygous). *Synkaryon division* (= postgamic nuclear division): mitosis of the synkaryon without cell division. *Vegetative (old) macronucleus*: macronucleus of vegetative cells. *Ventral-to-dorsal* (oral bulge) union mode: the oral bulge ends of the partners are directed oppositely; the brush of only one partner is visible if specimens are observed in the same focal plane (Fig. 44, 45).

RESULTS

Morphology of vegetative specimens. The vegetative cells matched previous descriptions of *Protospathidium serpens* (Kahl, 1930) Foissner, 1981 (Foissner 1996). Thus, mainly features relevant to the present study will be briefly described. The body size was about 69 μm × 13 μm with a length:width ratio of 5.4:1 in protargol preparations (Table 1). The macronucleus

Table 2. Frequency of macronuclear anlagen in spathidiids from non-flooded Petri dish (raw) cultures.

Species/population	Pairs (%)	Specimens with various macronuclear (MA) anlagen (%)[c] with 4 MA	with 2 MA	other patterns	Specimens analyzed, number[b]	References
Apertospathula armata from Greece	11	0	40	9 (3 MA)[d]	35	this paper
Apertospathula inermis from USA	0	~17 (with two to five globules)			30	Foissner et al. (2002)
Arcuospathidium atypicum from Australia	0	occur	?	? (3 MA)	30	Foissner (1988)
Arcuospathidium japonicum from Japan	0	occur	?	? (1 or 3 MA)	~20	Foissner (1988)
Arcuospathidium muscorum from Austria	0	?	common	?	>20	Foissner (1981)
Arcuospathidium pelobium from Austria	0	frequently with 2 to 4 MA nodules			~20	Foissner & Xu[a]
Arcuospathidium vermiforme from Austria	0	0.3	?	10 (1 MA)[e]	311	Foissner (1984)
Cultellothrix tortisticha from Brazil	0	0.8	0	? (1 MA)	121	Foissner & Xu[a]
Edaphospathula inermis from Tibet	0	10 (with 2 or 4 MA)		0	50	Foissner & Xu[a]
Edaphospathula paradoxa from Austria	0	31	12	0	70	Foissner & Xu[a]
Epispathidium ascendens from Austria	8	36	4	8 (1, 3, 6 MA)	108	this paper
Protospathidium fusioplites, Stampfltal, Austria	0	9	6	0	40	Foissner et al. (2004)
Protospathidium fusioplites, Burgenland, Austria	0	16 (with 2 or 4 MA)		0	74	Foissner & Xu[a]
Protospathidium serpens from South Africa[f]	16	66	1	4 (3, 5, 6, 8 MA)	161	this paper
Protospathidium vermiculus from Iceland	0	5 (with 1 to 4 nodules)			77	Foissner & Xu[a]
Spathidium spathula from USA[f]	occur	typical	occur	?	many	Woodruff & Spencer (1924)
Spathidium turgitorum from Namibia[f]	0	1 (3 to 6, usually 4 MA)			19	Foissner et al. (2002)

[a] Several names are not yet available, that is, will be published in a monograph on spathidiids (Foissner and Xu 2005).
[b] Vegetative and pairing cells, as well as specimens with various macronuclear anlagen.
[c] Exconjugants, exautogamonts, or freshly excysted cells.
[d] Among three macronucleus nodules at least one shows distinct signs of disintegration.
[e] About 8.4% of specimens possess a rod-shaped nucleus, and 2.2% a globular nodule.
[f] From pure or semi-pure cultures.

was invariably a more or less tortuous, nodulated strand, while highly variable in most other populations (Foissner 1981, 1996), typically accompanied by two to five, usually three ($\bar{x}$ 2.9; n = 13) micronuclei about 2 μm across. Extrusomes were slightly curved, about 4-μm long rods with inconspicuously narrowed ends, and impregnated with the protargol method used. There were on average 12 somatic ciliary rows (n = 11), three anteriorly differentiated to a dorsal brush located slightly dorsolaterally: about seven dikinetids in brush row 1, 11 in row 2, and eight in row 3 (n = 5); dikinetids ordinarily spaced in main kinety axis. The temporary cytostome (mouth s. l.) was a minute cone in or near the center of the obovate oral bulge. The discontinuous circumoral kinety was composed of short, dikinetidal kinetofragments, as typical for the genus. Descriptions of *E. ascendens* and *A. armata* are found in Foissner (1987b) and Foissner, Agatha, and Berger (2002).

Conjugation. Among 161 randomly selected specimens (Table 2), 13% had a tortuous, more or less nodulated macronucleus strand and were thus vegetative cells; 16% were conjugation pairs; and 71% were exconjugants, that is, usually had four macronuclear nodules (66%), rarely two nodules (1%), or three, five, six, or even eight nodules (4%). Thus, there was mass conjugation about 10 h before the culture was fixed. Although conjugation is a continuous process, we classify it, for the sake of clarity, into five stages mainly based on distinct nuclear events.

Pair formation (Table 1 and Fig. 1–18, 34–37). Pair formation is heteropolar, and the partners unite with the oral bulge. However, the exact way is difficult to observe because the bulge is minute and roundish (Fig. 34–37). However, all pairs were united in such a way that the dorsal brush was *fully* recognizable only in one cell. Thus, union cannot be exactly dorsal-to-dorsal. It is also not ventral-to-dorsal because the brushes were not fully opposite. Only if the partners united obliquely is the pattern recognizable in the slides obtained (Fig. 36, 37). The exact angles are difficult to determine because they depend on the angles the cells were viewed (Fig. 34–37). Likewise, plasticine models showed that it is impossible to decide whether union is basically dorsal-to-dorsal or ventral-to-dorsal.

The conjugants may form a rod-like or strongly arched pair (Fig. 1–4, 6–18). Among a total of 15 early conjugants investigated, 47% were rod-shaped or slightly arched (Fig. 1, 2, 4), while 53% were arched by 30° to almost 180° (Fig. 3). The same proportion was recognizable in 34 late conjugants. Thus, there is no correlation between pair shape and progress through conjugation.

Partners of early conjugants have the same size as vegetative cells, but commence to broaden, as shown by the decreased length:width ratio (Table 1). Thus, the length similarity is consistent with the absence of a preconjugation division.

The vegetative macronucleus loses its nodular appearance and commences condensation from the distal ends, as indicated by their slight inflation. The micronuclei appear unchanged (Fig. 1). Two deviating cases were observed: in the first pair, one of the partners performed asexual cell and nuclear division, while the micronuclei of the other partner were in an early prophase stage (Fig. 2); in the second pair, one partner had two condensed macronuclear masses, likely due to a separated vegetative nucleus.

Maturation divisions (Table 1 and Fig. 3, 4, 6–10). Three maturation divisions occur. They were associated with distinct changes in body size, body shape, and the appearance of the nuclear apparatus. The changes became more pronounced during the second and third maturation divisions.

The body distinctly shortened and broadened from 69 × 13 μm to 40 × 19 μm, that is, became ellipsoidal on average.

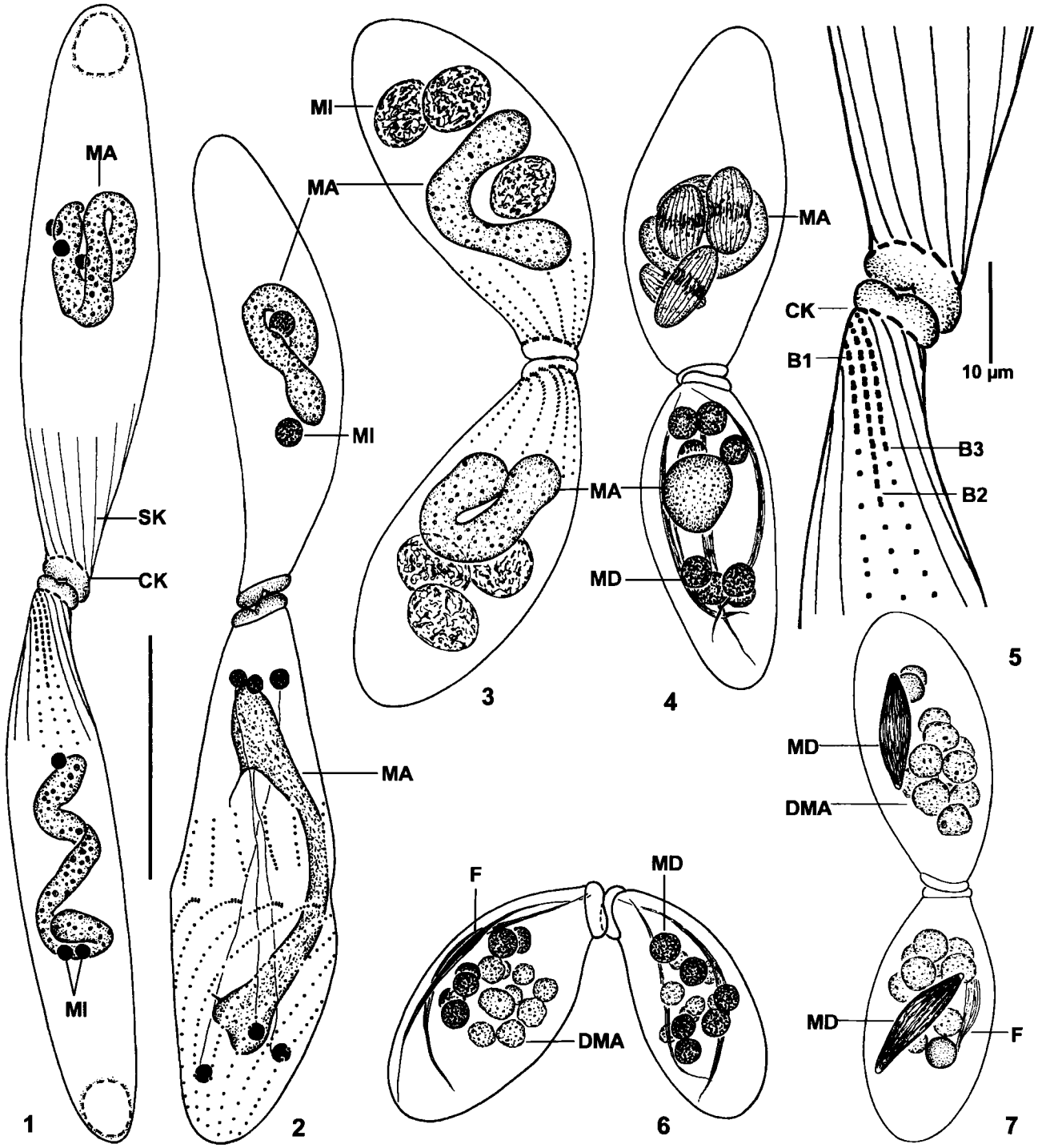

Fig. 1–7. *Protospathidium serpens*, protargol-impregnated early and maturing conjugants. **1, 5.** Very early conjugant. The partners are of same size as vegetative cells. The macronucleus commences to condense and the micronuclei are in interphase to early prophase. **2.** A deviant, early conjugant with one partner performing asexual division. **3.** Prophase of the first maturation division. **4.** Metaphase in the anterior and telophase in the posterior partner of the second maturation division. The macronucleus condensed to a globular mass. **6.** Very late telophase of the second maturation division. The macronucleus disintegrates into many globules. **7.** Anaphase of the third maturation division. Note the fusiform maturation derivative. B1–3, dorsal brush rows; CK, circumoral kinety; DMA, disintegrating vegetative macronucleus; F, rest of division spindle; MA, vegetative macronucleus; MI, micronuclei; MD, maturation derivatives; SK, somatic kineties. Fig. 1–4, and 6–27 drawn to scale, bar = 30 µm.

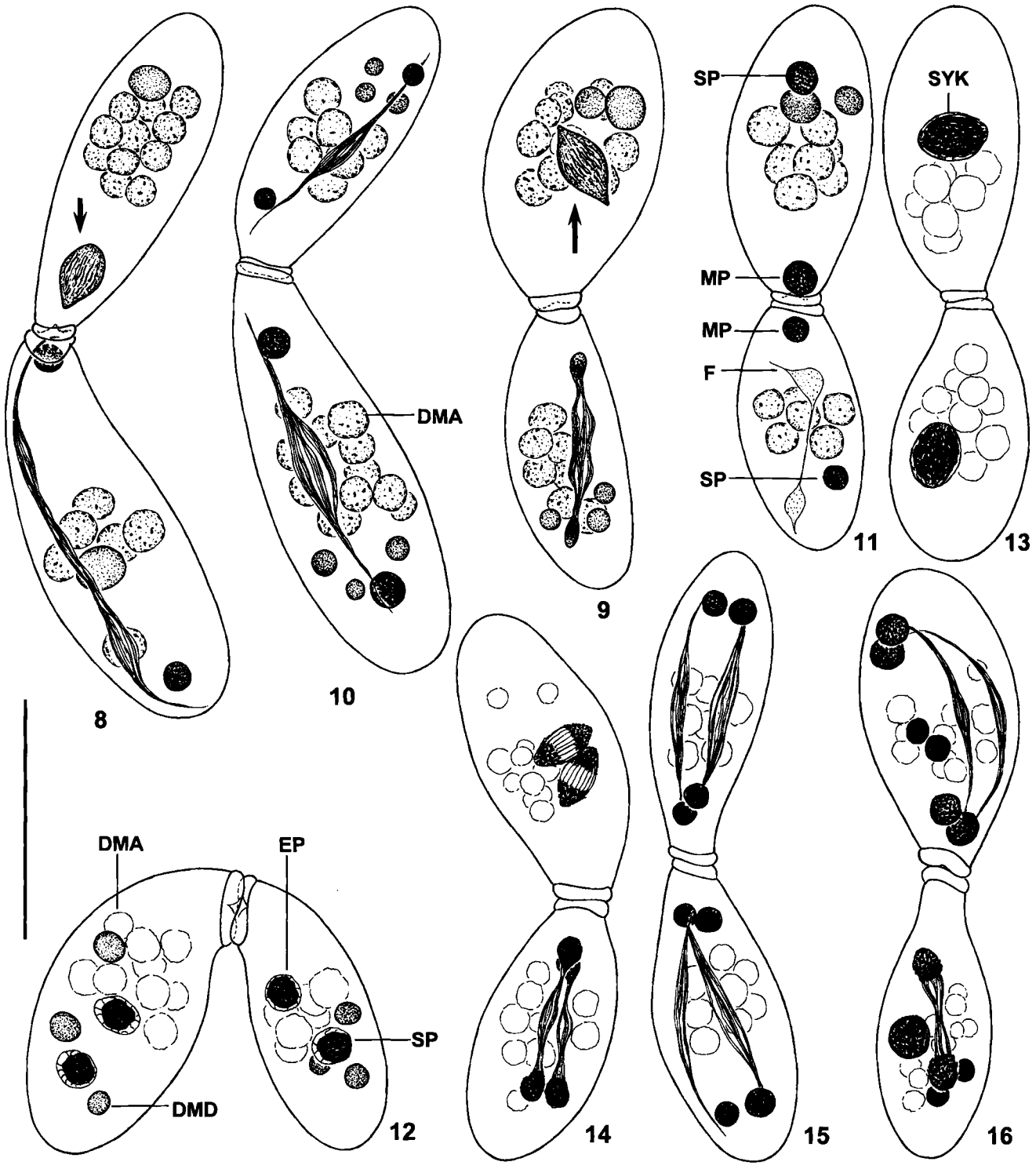

Fig. **8–16.** *Protospathidium serpens*, protargol-impregnated, maturing and synkaryon developing conjugants. **8–10.** Metaphase to telophase of the third maturation division. Arrows denote the characteristic, fusiform to lanceolate maturation derivative. **11.** Pronuclei formation. **12.** The migratory pronucleus approaches the partner's stationary pronucleus in prophase. **13.** Synkaryon formation. The synkaryon is broadly ellipsoidal in prometaphase of the first division. **14, 15.** Anaphase to telophase of the second synkaryon division. **16.** Telophase of the third synkaryon division. Only two synkaryon derivatives each undergo the third division generating four macronuclear anlagen; the two non-dividing derivatives become micronuclei. DMA, disintegrating vegetative macronucleus; DMD, degenerating maturation derivative; EP, exchanged pronucleus; F, rest of division spindle; MP, migratory pronucleus; SP, stationary pronucleus; SYK, synkaryon. Drawn to scale, bar = 30 μm.

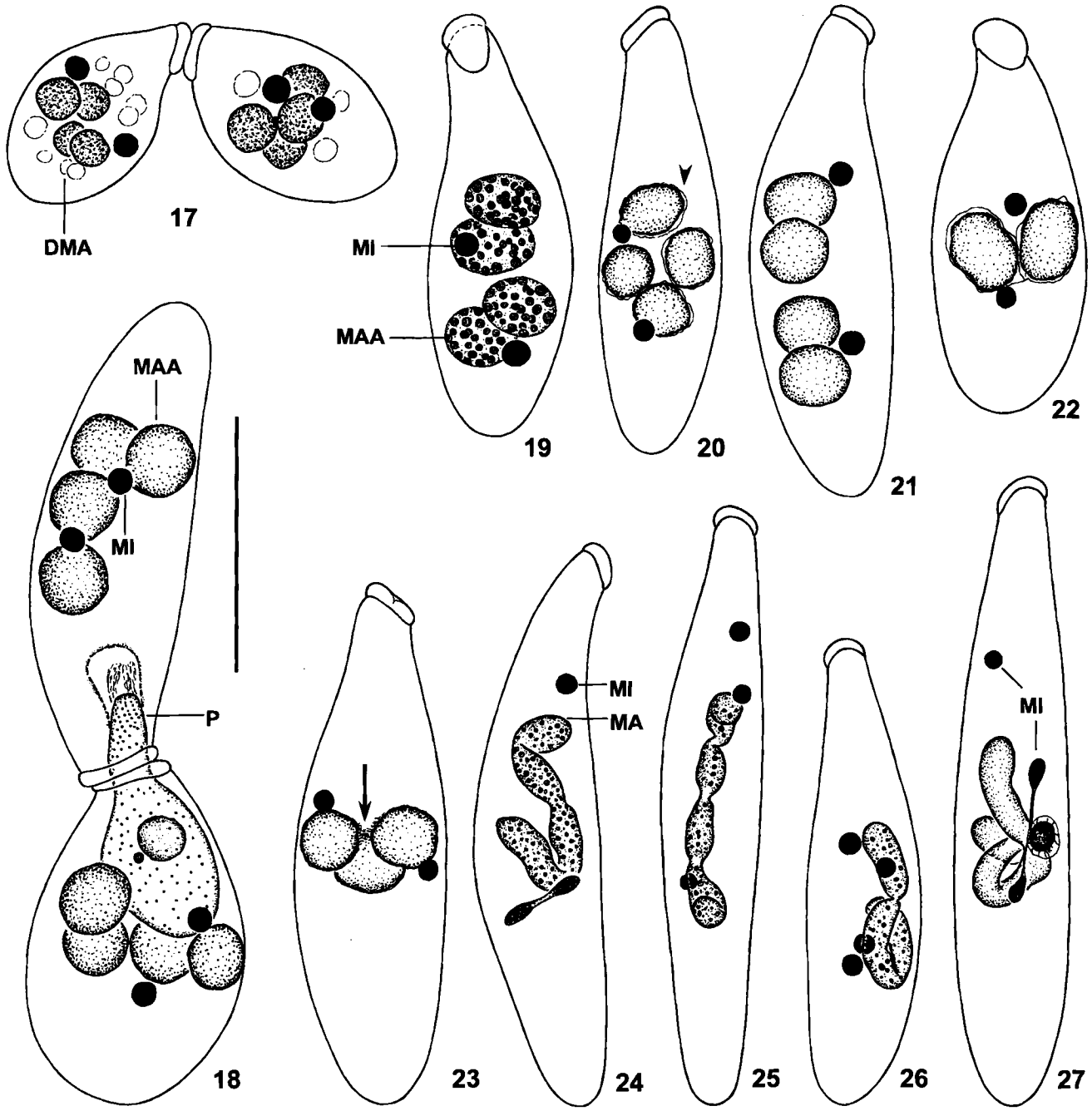

Fig. **17–27.** *Protospathidium serpens*, late conjugants and exconjugant nuclear reconstruction in protargol preparations. **17.** A very late conjugant. The pairs separate after this stage, and thus early exconjugants have a very typical nuclear apparatus, viz., four macronuclear anlagen and two micronuclei. **18.** Bilateral reconjugation (see text). **19.** A very early exconjugant, as shown by the small and stout body. **20, 21.** Two exconjugants each with the typical four macronuclear anlagen and two micronuclei. The macronuclear anlagen grow to 8–9 μm and intensely impregnate with protargol. The membrane surrounding each macronuclear anlage is frequently wrinkled and rather distinctly separate from the nucleoplasm (arrowhead). **22, 23.** Exconjugants with two or three macronuclear anlagen, which are generated by stepwise fusion of the four macronuclear anlagen. Arrow marks some curious fibers between anlagen. **24–27.** Very late exconjugants with the macronucleus strand composed of four more or less distinct, ellipsoidal nodules, that is, the former four macronuclear anlagen. The micronuclei may undergo further division. Exconjugant division does not occur. DMA, disintegrating vegetative macronucleus; MA, vegetative macronucleus; MAA, macronuclear anlagen; MI, micronuclei; P, prey. Drawn to scale, bar = 30 μm.

Usually, the partners had only slightly different size, but the size differences were rather pronounced in about one-third of the specimens (Fig. 8). Thus, we performed detailed measurements to determine whether conjugation of *P. serpens* is isogamic or anisogamic (Table 1): vegetative cells had a coefficient of variation for length of 11% (68.9 ± 7.6 μm), while the variability of maturing cells was slightly higher, as expected: 13.8% and 11.4% (49.8 ± 6.9 μm, 44 ± 5 μm). Taking into account the variability and the rather small average differences (≤ 6 μm), it is likely that conjugation is isogamic. This con-

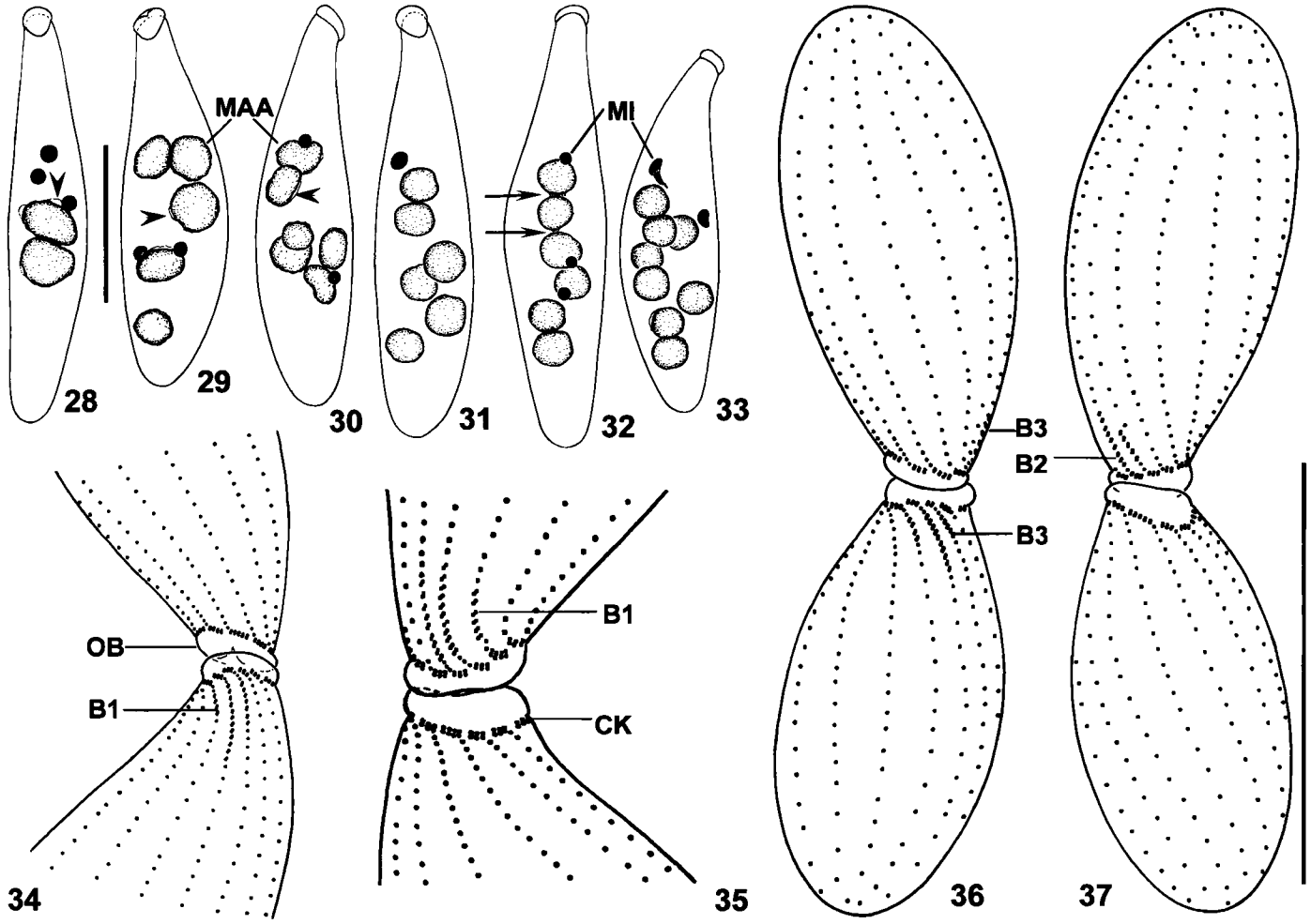

Fig. **28–33.** Conjugation of *Protospathidium serpens*. Fig. **28–33.** Exconjugant nuclear reconstruction in protargol preparations. Arrowheads denote the wrinkled membrane around each macronuclear anlage. Arrows indicate fibers between anlagen. **28.** An exconjugant with two macronuclear anlagen and three micronuclei. **29–33.** Exconjugants with five to eight macronuclear anlagen and one to three micronuclei. Five, six or eight macronuclear anlagen, instead of the usual four, are likely produced by one or both progenitor micronuclei. MAA, macronuclear anlagen, MI, micronuclei. Drawn to scale, bar = 30 μm.

Fig. **34–37.** Ciliary pattern of the protargol-impregnated conjugants shown in Figures 3 and 7. Pair formation is heteropolar, and the partners unite obliquely with the oral bulge. Thus, the dorsal brush is fully recognizable only in one partner. We could not clarify whether union is dorsal-to-dorsal or ventral-to-dorsal because the bulge is minute and roundish. Conjugation does not cause basic changes in the ciliary pattern, though some adjustments occur, for instance, the dorsal brush dikinetids become obliquely arranged due to the distinct body shortening. B1–3, dorsal brush rows; CK, circumoral kinety; OB, oral bulge. Scale bar = 30 μm.

clusion is supported by the cell dimensions in the synkaryon stage, where the partners had almost same length (Table 1).

In maturing specimens, the oral bulge appears more roundish, but changes are indistinct and cannot be quantified because the bulge is minute and obovate. The food vacuoles disappeared before the formation of the pronuclei, making the cells more transparent.

As concerns the nuclear apparatus, the onset of the first maturation division is characterized by a conspicuous swelling of the micronuclei from a diameter of about 2 μm to 10 μm. The swollen micronuclei impregnate faintly and show a fibrous structure, presumably due to the despiralizing chromosomes (Fig. 3). This and the following division leave conspicuous fibrous division spindles, which are usually resorbed before the next division commences. During the second maturation division, each micronucleus divides again, showing more than 20 chromosomes in the ellipsoidal to dumbbell-shaped metaphase maturation derivatives (Fig. 3, 4, 6). Of the many maturation derivatives now present, all degenerate slowly and impregnate homogenously, except for one, which enters the third maturation division and generates the pronuclei (Fig. 7–10). This single maturation derivative is usually located in or near mid-body, rarely in the anterior or posterior body end; it assumes a highly characteristic fusiform, rarely lanceolate shape, during metaphase and anaphase (Fig. 7–9).

The vegetative macronucleus condenses to a globular mass and disintegrates into about 10 globules after the second maturation division. The globules impregnated more lightly than the maturation derivatives, but are difficult to count because they were concentrated in the middle body third (Fig. 6).

Pronuclei and synkaryon formation (Table 1 and Fig. 11–13, 38). No further changes occurred in body shape and size at this stage (Table 1), but the mouth opening (i.e. the cytostome s. str.) appeared more or less distinctly enlarged (Fig. 11,

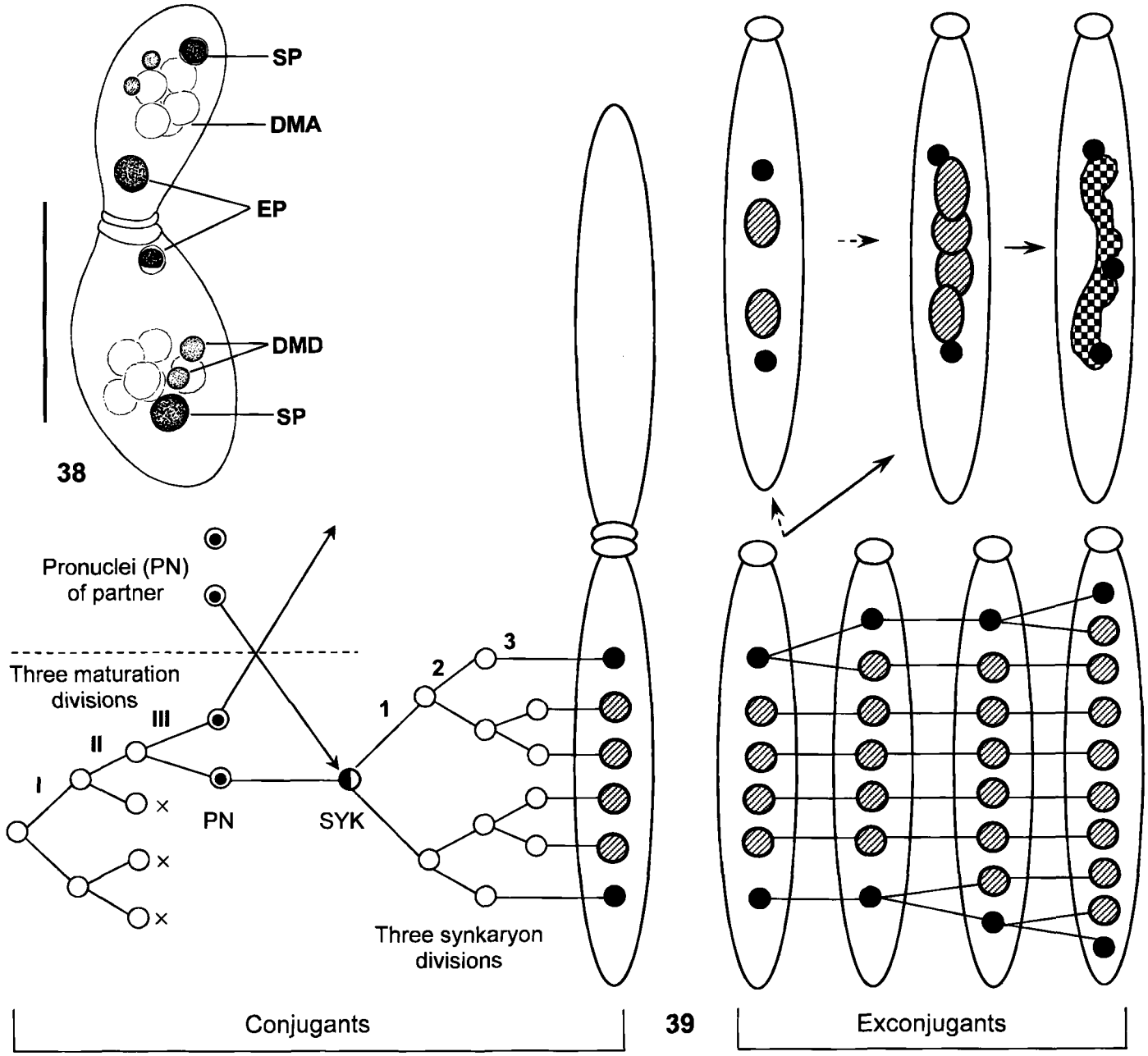

Fig. 38. *Protospathidium serpens*, a protargol-impregnated conjugant after exchange of pronuclei. The sizes and structure of the partner's pronuclei are different, indicating that each partner contains a pronucleus from the other partner. DMA, disintegrating vegetative macronucleus; DMD, degenerating maturation derivatives; EP, exchanged migratory pronuclei; SP, stationary pronuclei. Scale bar = 30 μm.

Fig. 39. Scheme of the nuclear processes during conjugation of *Protospathidium serpens* (shown for one of the three micronuclei). There are three maturation divisions and three synkaryon divisions, which produce four macronuclear anlagen and two micronuclei. Arrows in exconjugants denote the usual way to form the vegetative macronucleus, that is, by fusion and elongation of the four macronuclear anlagen. Dashed arrows indicate that the four anlagen may fuse into two nodules first and then a strand. Further divisions of the micronuclei may generate supernumerary macronuclear anlagen in exconjugants. I, II, III, maturation divisions of the micronucleus; 1, 2, 3, synkaryon divisions; PN, pronuclei; SYK, synkaryon. In the conjugants and exconjugants, circles are nuclear derivatives of divisions and black dots are micronuclei; hatched circles are macronuclear anlagen. Crosses mark degenerating nuclei.

12). Soon the spindle fibers of the third maturation division degenerated, but occasionally some residues were recognizable.

As described above, the derivative undergoing the third maturation division yielded two identical globular pronuclei in each partner: the one in the posterior body end was stationary, the other near the junction was migratory (Fig. 11). The size of the partner's migratory pronuclei may be slightly different (on average 4 μm vs. 3 μm), depending on their developmental state and that of the partner: usually, the larger pronucleus was nearer to the junction (Fig. 11). Then the migratory pronuclei were exchanged, as shown by a fortunate pair with each partner obviously containing a pronucleus from the other partner, as ev-

ident from the size and structure of the pronuclei (Fig. 38). Occasionally, the transfer of a migratory pronucleus was delayed, resulting in three pronuclei in one partner and only one in the other. The migratory pronuclei approached the partner's stationary pronucleus in prophase state (Fig. 12), and the synkaryon was thus formed by fusion of the pronuclei (Fig. 13). The degenerating maturation derivatives and the resorbing vegetative macronuclear globules were still clearly recognizable, most disappearing before separation of the conjugants (Fig. 12).

Synkaryon divisions and pair separation (Table 1 and Fig. 13–17, 19–21). The three synkaryon divisions were associated with further body diminution, which reached a minimum in late conjugants with four macronuclear anlagen (Table 1). However, very small pairs occurred also at the beginning of the synkaryon division.

The synkaryon was broadly ellipsoidal during prometaphase and metaphase and was thus easily distinguishable from the lenticular maturation derivative undergoing the third division (Fig. 7–9, 13). The synkaryon divided mitotically three times. The first division immediately followed the pronuclear fusion and yielded two synkaryon derivatives very near to each other (Fig. 14). The second division produced four synkaryon derivatives in each partner, viz., two each near the anterior and two near the posterior body end (Fig. 15). Of the four synkaryon derivatives, only two entered the third division and produced four macronuclear anlagen, which gradually increased in size; the two non-dividing derivatives condensed to micronuclei about 3 μm across (Fig. 16). The individual macronuclear anlagen were on average 5 (4–6) μm across in late conjugants, and each contained many minute (<1 μm) granules (Fig. 17).

The resorption of the disintegrating vegetative macronuclear globules was obviously slow because they were still recognizable in very late pairs (Fig. 17). Likely, the resorption is completed soon after separation of the conjugants because the globules were absent in four very early exconjugants.

The conjugants separated after the third synkaryon division. Very early exconjugants were recognizable by their small size and the very typical nuclear apparatus (viz. four macronuclear anlagen and two micronuclei). Pair separation was not associated with visible changes of the oral bulge and infraciliature (17, 19–21).

Exconjugant nuclear reconstruction (Table 1 and Fig. 18–33, 39). On average, early exconjugants were distinctly larger and more slender than the partners of late conjugation pairs because they are able to feed soon after separation (Table 1). Feeding was demonstrated by fresh food vacuoles in 25 out of 32 very early and early exconjugants; seven specimens were even well-fed. True growth was indicated by the increasing length:width ratio and the minimum body length values, which were considerably higher in early exconjugants than late pairs (Table 1). Vegetative body size was reached only in late exconjugants with fusing macronuclear anlagen. Notably, vegetative body length was reached earlier than vegetative body width (Table 1).

The exconjugant macronuclear anlagen grew to 8–9 μm and all, except for very early stages, impregnated intensely with protargol (Fig. 20, 21). Each macronuclear anlage was surrounded by a more or less wrinkled membrane slightly apart from the nucleoplasm; whether or not this space and the wrinkles are preparation artifacts needs further investigations (Fig. 20, 22, 28–30). The two micronuclei were slightly larger than those of vegetative cells.

Usually, the four macronuclear anlagen became ellipsoidal and then fused to a nodulated strand. Thus, late exconjugants often had a macronuclear strand composed of four more or less distinct, ellipsoidal nodules (Fig. 24–27). Occasionally, the macronuclear anlagen fused stepwise, that is, first to three (Fig. 23) and then to two nodules (Fig. 22, 28). The micronuclei may undergo further divisions during this process (Fig. 24–28). No dividers were found among more than 100 early (with four macronuclear anlagen) and late (two or three or more than four macronuclear anlagen) exconjugants. Thus, exconjugant division does not occur in *P. serpens*.

Occasionally, five, six or even eight macronuclear anlagen occurred (Table 2 and Fig. 29–33). Probably, they were produced by one or both progenitor micronuclei, as indicated by an exconjugant with eight macronuclear anlagen and two just divided micronuclei (Fig. 33). Whether such specimens survived is not known.

Bilateral reconjugation was observed in one pair (Fig. 18). Each partner had four macronuclear anlagen and two micronuclei, as usual in late conjugants. However, the cells were full of food vacuoles and one partner showed a just captured *Cyrtolophosis mucicola*. Furthermore, the cells were 42–56 μm long (vs. about 30–34 μm; Table 1), the macronuclear anlagen were 8–9 μm across (vs. about 5 μm in late conjugants), and disintegrating vegetative macronuclear nodules were absent (vs. present in all five late conjugants found). The fate of reconjugants is not known (Raikov 1972).

Ciliary pattern (Fig. 1–3, 5, 34–37). Conjugation did not cause basic changes in the ciliary pattern. However, some adjustments occurred in connection with alterations in the body shape and size as described above: the circumoral kinety became more or less distinctly circular, the interkinetal distances increased, while the distances between the basal bodies decreased due to body diminution. Body diminution also caused the narrowly spaced brush dikinetids to become obliquely arranged. All changes disappeared as soon as the exconjugants assumed the vegetative cell shape and size. The nematodesmata were not apparently resorbed.

Observations on *Epispathidium ascendens* and *Apertospathula armata*. To get a broader view, we performed a preliminary study of *E. ascendens* and *A. armata*. Both species have good markers for determining the union mode. *Epispathidium ascendens* has an oblong oral bulge with a conical mouth opening near the dorsal end. *Apertospathula armata* has the circumoral kinety shortened on the left side of the oral bulge and dorsal brush row 3 has a monokinetidal tail composed of two bristles.

These investigations revealed an unexpected diversity in the union mode of spathidiids. *Epispathidium ascendens* unites mouth-to-mouth with the dorsal portion of the oral bulge. The union is oblique and in the ventral-to-dorsal mode (Fig. 40–42). Thus, distinct "steps" were formed (Fig. 40, 41), which later disappeared when the bulge became circular and the mouth larger and concave (Fig. 42). *Apertospathula armata*, in contrast, shows three distinct union modes: exactly dorsal-to-dorsal in half of eight pairs investigated (Fig. 43), exactly ventral-to-dorsal (Fig. 44, 45) or obliquely (Fig. 46) in two pairs each.

The nuclear processes are also rather diverse and will be described later. Briefly, *E. ascendens* likely has preconjugation division, and the exconjugant nuclear pattern (four macronuclear anlagen and two micronuclei) is generated as follows: three synkaryon divisions produce eight synkaryon derivatives, four of which become macronuclear anlagen and two become micronuclei, while two degenerate. In contrast, *P. serpens* lacks preconjugation division and the three synkaryon divisions produce only six synkaryon derivatives, four of which become macronuclear anlagen and two micronuclei. Only a few stages could be observed in *A. armata*; it is thus difficult to determine whether they represent ordinary conjugation or autogamy (in pairs), two ways of nuclear reorgani-

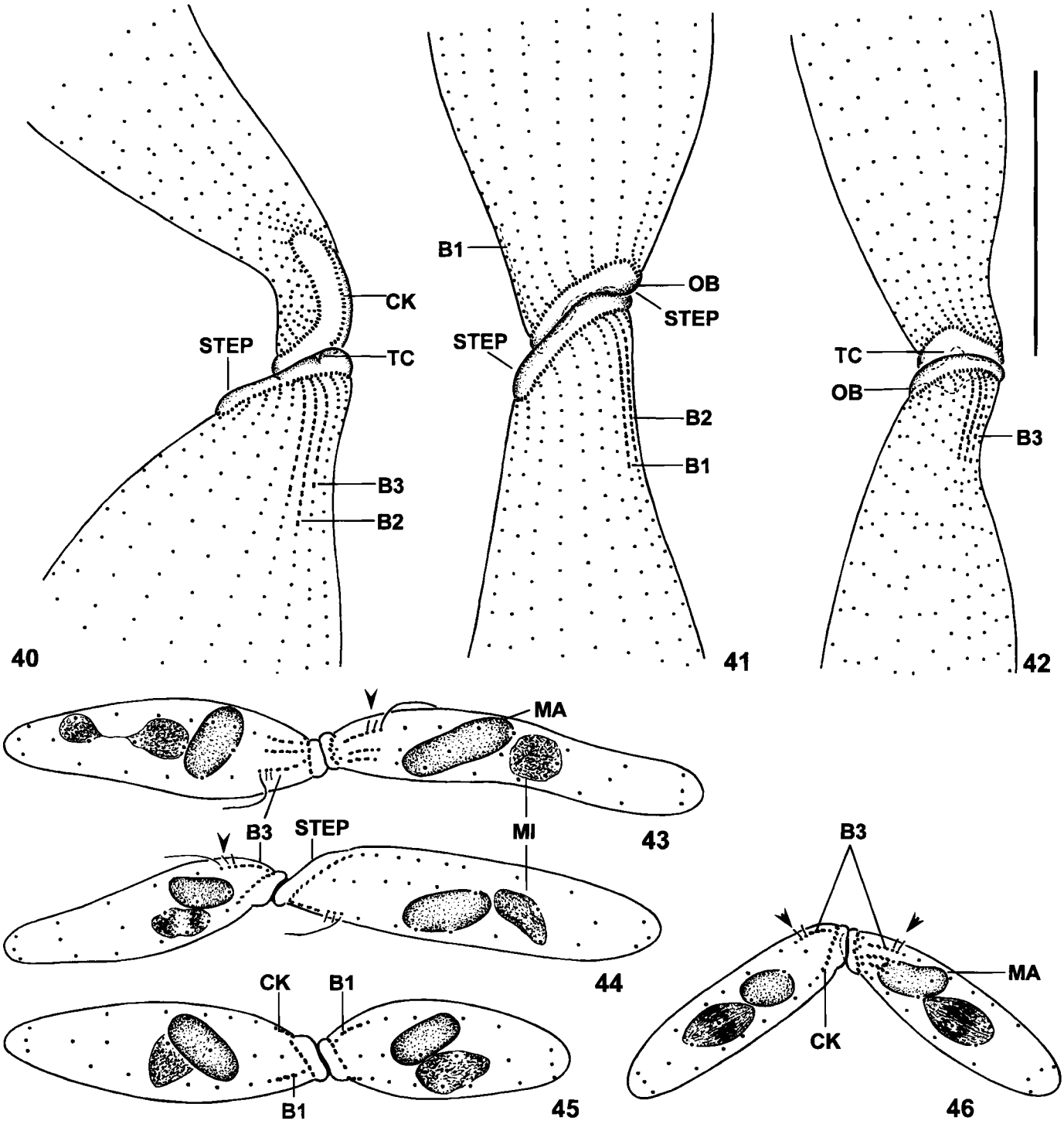

Fig. **40–46.** Conjugating *Epispathidium ascendens* (40–42) and *Apertospathula armata* (43–46) in protargol preparations. **40–42.** Ciliary pattern in the oral region of three early pairs. *Epispathidium ascendens* unites mouth-to-mouth with the dorsal portion of the oral bulge (40). The union is oblique and in the ventral-to-dorsal mode. Thus, distinct "steps" are formed (40, 41), which later disappear when the bulge becomes circular and the mouth larger and concave (42). **43–46.** *Apertospathula armata* unites also mouth-to-mouth with the oral bulge, but shows three distinct union modes: exactly dorsal-to-dorsal (43), exactly ventral-to-dorsal (44, 45), and oblique (46). Arrowheads denote the monokinetidal bristle tail of brush row 3. The vegetative macronucleus disintegrates and the micronucleus is in maturation divisions. Further details, see text. B1–3, dorsal brush rows; CK, circumoral kinety; MA, vegetative macronucleus; MI, micronucleus; OB, oral bulge; TC, temporary cytostome. Drawn to scale, bar = 30 μm.

Table 3. Conjugation patterns in spathidiids and related haptorid ciliates.

Characteristics	*Protospathidium serpens*	*Spathidium spathula*	*Acaryophyra collaris*	*Didinium nasutum*	*Dileptus gigas* sensu Visscher (1927)[f]	*Dileptus anser*
Type of conjugation	temporary	temporary	temporary	temporary	temporary	temporary
Isogamic or anisogamic	isogamic	likely isogamic	likely isogamic	likely isogamic	isogamic	likely isogamic
Preconjugants	no	no	?	yes, distinctly smaller	yes, distinctly smaller	yes
Pair formation (of body)	heteropolar	heteropolar	heteropolar	heteropolar	heteropolar	heteropolar
Union mode (of oral bulge)	oblique	dorsal-to-dorsal	oblique	the proboscis ends fuse	ventral-to-dorsal[g]	ventral-to-dorsal[g]
Body becomes smaller and broader	yes	yes	?	?	yes	yes
Ciliary changes	no	?	no	?	?	yes[h]
Micronuclei undergoing maturation divisions, number	all	?	the only one	the only one	only one of the many	almost all
Maturation divisions, number	3	?	3	3	3	3
Maturation derivatives undergoing third maturation division, number	1	?	1	1	1	1
Difference between pronuclei	no	?	different shape	different shape	no	different size
State of pronuclei during fusion	in prophase	?	?	likely in prophase[a]	?	?
Synkaryon divisions, number	3	?	3	2	1	1 to 4[i]
Synkaryon derivatives undergoing a third synkaryon division, number	2	?	3	no	no	?
Degenerating synkaryon derivatives	no	?	one	no	no	?
Macronuclear anlagen, number	4	4	4	2	1	1 to 11[j]
New micronuclei, number	2	several	2	2	1	1 to 4
Behavior of macronuclear anlagen	fusion[b]	segregation[c]	segregation	fusion or segregation[d]	divisions/fragmentation[e]	division
Pair separation	after formation of macronuclear anlagen	after formation of macronuclear anlagen	after the first synkaryon division	?	after formation of synkaryon	?
Exconjugant divisions	no	two	yes	may occur	occasionally occur	no
Vegetative (old) macronucleus	fragmentation in middle body third after condensation	?	pycnosis without fragmentation	fragmentation without preceding skein formation or condensation	migration to body end; pycnosis without fragmentation	pycnosis without fragmentation
References	this paper	Woodruff & Spencer (1924)	Serrano et al. (1990)	Prandtl (1906)	Visscher (1927)	Vinnikova (1974)

[a] In interphase (vesicular) or spindle form (more or less metaphase) according to Raikov's (1972) interpretation.
[b] No exconjugant division, and thus the macronuclear anlagen fuse to a single macronucleus.
[c] The individual macronuclear anlagen are distributed among daughter cells during exconjugant division(s).
[d] Five ways of nuclear reconstruction occur in *D. nasutum*. Fusion is the most frequent way, segregation is facultative; the latter guides exconjugant divisions.
[e] Division of a single anlage into many macronucleus nodules, some or all of which then break up into numerous granules smaller than micronuclei.
[f] Likely misidentification.
[g] This is sustained by observations of Golinska and Afon'kin's (1993).
[h] Shown by Golinska and Afon'kin (1993).
[i] Curious variation because the number of synkaryon divisions is rarely variable within a certain species.
[j] Curious because the number of macronuclear anlagen does not vary within species; however, some exceptions have been reported.

zation cytologically almost identical, except for the exchange of pronuclei during conjugation. Nonetheless, the occurrence of only two macronuclear anlagen and one micronucleus indicate that the nuclear processes are different from those of the afore-mentioned species.

DISCUSSION

Data on conjugation of haptorids are very scant, and our study is the first to concomitantly investigate changes in cell size, cell shape, nuclei, and kinetome (Table 3). Basically, con-

jugation of *P. serpens* follows the usual mode, excellently reviewed by Raikov (1972). Thus, we restrict the discussion to a few main issues and refer to Table 3 for details.

Pair formation and union mode are rather diverse in haptorid gymnostomes (Table 3). Pair formation is usually heteropolar and mouth-to-mouth, but the pleurostome haptorids (e.g. *Litonotus*) form homopolar pairs and fuse along the main body axis (Kahl 1930). Union may be oblique, for instance, in the spathidiids investigated and in *Acaryophrya collaris* (Serrano, Martín-González, and Fernández-Galiano 1990); dorsal-to-dorsal, as in *Apertospathula armata* and *Spathidium spathula* (Fig. 43; Table 3); or ventral-to-dorsal, for instance, in *Dileptus* (Golinska and Afon'kin 1993) and part of the *Apertospathula* pairs (Fig. 44, 45). Homopolar pair formation is more widespread than heteropolar pair formation (Golinska and Afon'kin 1993; Raikov 1972). Nonetheless, heteropolar pair formation is not specific to haptorids because it occurs, for instance, also in the Entodiniomorphida (Raikov 1972) and the chonotrichid *Spirochona* (Kahl 1930). Likewise, it is not associated with an apical mouth location because homopolar pair formation is found in the prostomatid *Urotricha* (Martín-González, Serrano, and Fernández-Galiano 1985) and in oligotrichs, e.g. *Strombidium* (Kahl 1930; Ota and Taniguchi 2003). Obviously, pair formation is unrelated to the systematic position of the species.

The exconjugation period is divided into four main types (I–IV) according to the number of synkaryon divisions and into sub-types according to the number and fate of the macronuclear anlagen (Dogiel 1925; Raikov 1972). Neither the main types nor the sub-types can be related to certain systematic categories or to other things. In *Paramecium* (Hymenostomata), for instance, the main types II, III, and IV, and many sub-types occur (Raikov 1972). Both *P. serpens* and *E. ascendens* have type III exconjugants and represent distinct sub-types (Fig. 39). In *P. serpens*, there is no exconjugant division and only two of the four synkaryon derivatives of the second synkaryon division enter the third division and generate four macronuclear anlagen, which fuse into a single macronucleus; further the two non-dividing derivatives become two micronuclei, which may continue division (Fig. 39). These processes resemble those of the sub-type *Climacostomum* (Heterotrichida) which, however, has four (vs. two) micronuclei originating from the third (vs. second) synkaryon division (Raikov 1972). *Epispathidium ascendens* is also a distinct sub-type because two of the eight synkaryon derivatives of the third synkaryon division degenerate.

Spathidiids with macronuclear anlagen occur in most field populations investigated (Table 2). Literature and the present study suggest that such specimens are either exautogamonts (Moore 1924a, b) or exconjugants. Nuclear reorganization during autogamy has not yet been investigated with modern techniques in spathidiids. The data from Moore (1924a, b) suggest that autogamy occurs, like in several other ciliates (Raikov 1972), just before encystment, in the resting cyst, or during and after excystment. This is sustained by our field data that showed a rather high percentage of transparent (non-feeding) specimens with two to six, usually four macronuclear pieces, while conjugating pairs were rare (Table 2). On the other hand, conjugation (pairing) is likely faster than exconjugant nuclear reconstruction and thus more difficult to recognize. For example, *Dileptus gigas* pairs for 8–12 h, but needs three to four days to complete nuclear reconstruction (Visscher 1927). Conjugation was often observed only during a definite season in wild ciliate populations (Raikov 1972). *Dileptus*, for example, conjugated only during spring (Visscher 1927). The trigger for the spontaneous mass conjugation in the cultures of *P. serpens* and *E. ascendens* is unknown.

The conjugation and endomixis (or autogamy) data are of high significance for alpha taxonomy of haptorids in general, and spathidiids in particular, because their nuclear pattern is frequently considered highly variable. However, if we do not consider the changes occurring in post-dividers (Foissner, Agatha, and Berger 2002), exconjugants (this paper), and excysting cells with endomixis (Moore 1924a, b), the nuclear pattern of the spathidiids is as constant (or variable) as that of other ciliates. The macronuclear anlagen stage is of special importance for alpha-taxonomy because, if it remains unrecognized, such specimens may be misidentified or even mistaken for distinct species with either two (Fig. 22, 28) or four (Fig. 21) macronuclear nodules or with a moniliform macronuclear strand (Fig. 24, 25, 32). Indeed, these patterns occur in several vegetative *Spathidium* s. l. species: *Arcuospathidium vermiforme*, for instance, has two macronuclear nodules (Foissner 1984), and *Spathidium moniliforme* has a moniliform macronucleus (Kahl 1930). Likewise, the distinct size reduction occurring, for instance, in preconjugant *E. ascendens*, may dramatically increase the variability of the population, if it remains unrecognized. For example, the body length of *Dileptus* decreases from 400 μm to 175 μm on average after two preconjugation divisions (Visscher 1927).

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