

## Morphology, Conjugation, and Postconjugational Reorganization of *Dileptus tirjakovae* n. sp. (Ciliophora, Haptoria)

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**ABSTRACT.** We studied the morphology, conjugation, and postconjugational reorganization of a new haptorian ciliate, *Dileptus tirjakovae* n. sp., using conventional methods. *Dileptus tirjakovae* is characterized by two abutting, globular macronuclear nodules and scattered brush kinetids. Conjugation is similar to that in congeners, that is, it is temporary, heteropolar, and the partners unite bulge-to-bulge with the proboscis. Some peculiarities occur in the nuclear processes: there are two synkaryon divisions producing four synkaryon derivatives, of which two become macronuclear anlagen, one becomes the micronucleus, and one degenerates. Unlike spathidiids, *D. tirjakovae* shows massive changes in body shape and ciliary pattern before, during, and after conjugation: early and late conjugants as well as early exconjugants resemble *Spathidium*, while mid-conjugants resemble *Enchelyodon*. These data give support to the hypothesis that spathidiids evolved from a *Dileptus*-like ancestor by reduction of the proboscis. *Dileptus tirjakovae* exconjugants differ from vegetative cells by their smaller size, stouter body, shorter proboscis, and by the lower number of ciliary rows, suggesting one or several postconjugation divisions. Although 83% of the exconjugants have the vegetative nuclear pattern, some strongly deviating specimens occur and might be mistaken for distinct species, especially because exconjugants are less than half as long as vegetative cells.

**Key Words.** Alpha taxonomy, evolution, exconjugants, Greece, soil ciliates.

**D**ILEPTID haptorids are characterized by having an oral opening underneath the proboscis (Corliss 1979; Foissner and Foissner 1988). They occur in a diverse variety of freshwater, marine, and soil environments, where they prey upon other ciliates and even microscopic metazoans, which are overwhelmed by the toxicysts contained in this ciliate's proboscis (Dragesco 1963; Foissner et al. 1995; Foissner, Agatha, and Berger 2002; Foissner, Berger, and Schaumburg 1999; Kahl 1931). Dragesco (1963), the last reviser of the genus *Dileptus* Dujardin, 1871, recognized 36 valid species and brought together many morphological and ecological data. Since then, further species have been described or redescribed, using detailed live observation, silver impregnation, and electron microscopy (Foissner 1984, 1989, 1997; Foissner et al. 2002; Song, Packroff, and Wilbert 1988; Song and Wilbert 1989).

More recently, molecular data classified the genus *Dileptus* as basal among the haptorids (Strüder-Kypke et al. 2006), partially supporting the hypothesis of Xu and Foissner (2005) that spathidiids evolved from a *Dileptus*-like ancestor by reduction of the proboscis. Our study adds a further indication for this: the formation of a *Spathidium*-like body shape and ciliary pattern during conjugation and postconjugational reorganization.

Details on sexual processes in *Dileptus* have been reported for only two species—*Dileptus anser* (Vinnikova 1974a, b, 1976) and *Dileptus gigas* (Hertwig 1904; Visscher 1927). However, the knowledge on somatic and ciliary changes during and after conjugation is scant because these studies did not use silver impregnation where both processes can be followed concomitantly. Therefore, we studied conjugation and postconjugational reorganization in a new *Dileptus* species, discovered in coastal soil from Greece.

### MATERIALS AND METHODS

**Collection data.** *Dileptus tirjakovae* n. sp. was discovered in soil taken in 1984 from the west coast of the Gulf of Nauplia, exactly opposite the town of Nauplia, Peloponnese, Greece. Here, the newly formed land was covered by a halophytic vegetation, especially, hemispherical, spiny colonies of rush. The soil was

strongly saline, very hard when dry, and had pH 6.9 in water. The sample consisted of vegetation residues and the upper 0–2 cm soil layer, which contained many fine grass roots. The material collected was air-dried for a month and then sealed in a plastic bag. After 6 months, one half of the well-mixed sample was used to set up a non-flooded Petri dish culture, as described by Foissner et al. (2002). *Dileptus tirjakovae* did not appear in this culture, but 10 years later, when the second half of the sample was treated as described before, it became abundant, feeding on bacilli (as recognizable by many spores in the food vacuoles) and medium-sized ciliates, such as peritrichs and metopids. Spontaneous, epidemic conjugation was noted 2 weeks after rewetting the sample.

**Methods.** Specimens were collected as described by Foissner et al. (2002), that is, the soil eluate was sampled, fixed in Stieve's solution, and impregnated with protargol protocol A (Foissner 1991). Live observation was performed as described in Foissner (1991). The conjugation process was reconstructed from 15 conjugation pairs found in eight protargol slides. They showed the whole process, but each stage was represented by only one to four pairs. Over 500 exconjugants were found in the slides, showing that we missed the peak of conjugation.

The illustration of the live specimen was based on free-hand sketches and represents a summary of the observations from live and prepared cells, as explained in Foissner et al. (2002). Figures from protargol-impregnated cells were made with a drawing device. Counts and measurements on impregnated specimens were performed at a magnification of 1,000X. In vivo measurements were conducted at magnifications of 40X to 1,000X. Morphometric data were computed according to statistics textbooks.

**Terminology.** Interphase terminology is shown in Fig. 1 and based on Corliss (1979) and, especially, Foissner and Xu (2007). Conjugation terminology is based on Grell (1968), Raikov (1972), Vinnikova (1976), and Xu and Foissner (2004).

The following terms are especially important: *conjugation division*, cell division in paired partners; *union mode oblique*, the partners unite more or less obliquely, usually the brush of both partners is partially recognizable; *union mode dorsal-to-dorsal*, the partners unite in a way that the dorsal and ventral ends of the oral bulge point in the same direction, and the brush of both partners is visible if specimens are observed in the same focal plane; *union mode dorsal-to-ventral*, the partners unite in a way that the oral bulge ends are directed oppositely, and thus the brush of only one partner is visible if specimens are observed in the same focal plane. These terms referring to the union mode need some expla-

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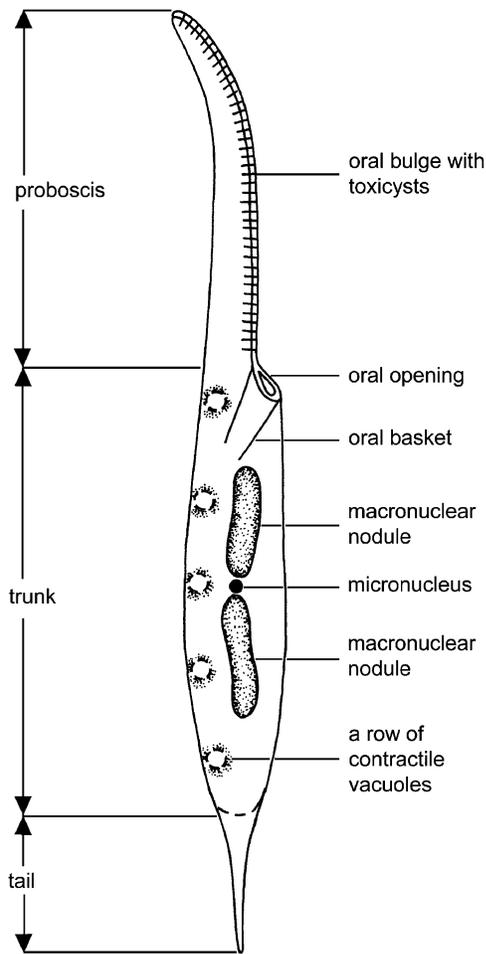


Fig. 1. Terminology and body organization of *Dileptus*.

nation. Basically, most haptorids fuse, like many other ciliates, with the oral area: the union mode is ventral-to-ventral (bulge-to-bulge). However, if the body is differentiated into a ventral and dorsal side, several union modes are possible, as explained by Xu and Foissner (2004), who used the dorsal brush as a specific marker. In *Dileptus*, which has a distinct ventral–dorsal differentiation, the union mode is ventral-to-ventral with respect to the oral bulge, but dorsal-to-ventral with respect to body and dorsal brush (Fig. 32, 35).

## RESULTS

**Description of *Dileptus tirjakovae* n. sp. (Tables 1 and 2 and Fig. 2–16).** The size is  $170\text{--}320 \times 15\text{--}35 \mu\text{m}$  in vivo, usually about  $230 \times 25 \mu\text{m}$ , as calculated from some in vivo measurements and the morphometric data, assuming 15% preparation shrinkage; the length:width ratio is highly variable (7.5–15:1) and on average near 10:1 in prepared cells (Table 1). This considerable variability could be partially caused by the inclusion of some preconjugants and/or very late exconjugants, although the coefficient of variation for body length is not unusually high (19%; Table 1). The shape is dileptid with conspicuous, slightly to distinctly curved proboscis occupying about 40% of body length; the dorsal outline is slightly to distinctly sigmoidal, and the specimens are usually widest in mid-body; the posterior end is elongate acute, very rarely tail-like (Fig. 2–10). The nuclear apparatus is slightly above mid of trunk. The macronucleus is highly

constant, consisting of two globular, abutting nodules in over 800 specimens analyzed; very rarely occur cells with either a single globular or ellipsoidal nodule (Table 2). The individual nodules are usually close together and about  $13 \mu\text{m}$  across in vivo; they contain many small, argyrophilic aggregates, possibly nucleoli (Fig. 2, 4–10). The micronucleus is usually in the vertex formed by the abutting macronuclear nodules, that is, it is not in between the nodules, a rare pattern found also in some other haptorids; it is globular, blister-like, and about  $3 \mu\text{m}$  across in vivo and deeply impregnated with protargol. A row with an average of six contractile vacuoles occurs on the dorsal side of the trunk and the proximal half of the proboscis; there are no ventral contractile vacuoles; each contractile vacuole has up to three excretory pores (Table 1; Fig. 2, 3). There are two types of extrusomes, which do not impregnate with protargol, attached to the broader right half of the oral bulge (Fig. 2, 12). Type I is rod-shaped to very narrowly ovate with rounded ends; it is about  $6 \times 1 \mu\text{m}$  in size and very sensitive, exploding partially and becoming more or less distinctly clavate under even slight coverslip pressure (Fig. 13). Type I extrusomes are frequent also in the cytoplasm, where certain developmental stages sometimes impregnate with protargol. Type II extrusomes are finely rod-shaped, about  $2.5\text{--}3 \mu\text{m}$  long, and more numerous than type I. The somatic cortex is very flexible and contains about 10 slightly oblique granule rows between adjacent kineties; the granules are inconspicuous because they are pale and only  $\sim 0.7 \times 0.3 \mu\text{m}$  in size. The cytoplasm is colorless, hyaline in the proboscis and the extreme posterior end, while opaque in the trunk due to numerous granules  $\sim 0.4 \mu\text{m}$  across and the  $3\text{--}10 \mu\text{m}$ -sized food vacuoles with sparse contents or some spores of bacteria, likely remnants from the prey. *Dileptus tirjakovae* feeds on medium-sized ciliates, such as *Metopus hasei* and *Vorticella astyliformis*, which are digested in large vacuoles often deforming the cells (Fig. 58–60).

The cilia are about  $8 \mu\text{m}$  long in vivo and ordinarily to densely spaced. In protargol preparations, they are as typical for dileptids: they have a thick, strongly impregnated distal half, except for the dorsal bristles. The cilia are arranged in an average of 21 longitudinal rows leaving a narrow, barren area left and right of the circumoral kinety (Table 1; Fig. 4, 5). The first row right of the oral bulge extends as the perioral kinety, which has closely spaced cilia along the circumoral kinety extending to the top of the proboscis. The dorsal brush is remarkable because it is not composed of staggered rows, as usual in dileptids, but of about 40 scattered dikinetids with clavate,  $3\text{-}\mu\text{m}$ -long bristles; the dikinetidal portion is followed by scattered monokinetids with  $1\text{-}\mu\text{m}$ -long bristles (Fig. 11, 14–16).

The oral apparatus is as in other dileptids. The oral opening is slightly above mid-body, and the base of the proboscis is only half as wide as the trunk, thus the pharyngeal opening projects distinctly. The pharyngeal basket is conspicuous both in vivo and in protargol preparations (Fig. 2, 4, 5). The circumoral kinety is dikinetidal, except for the monokinetidal portion around the oral opening. The preoral kineties are composed of two, rarely three monokinetids distinctly inclined to the circumoral kinety (Fig. 5).

**Conjugation (Fig. 17–35, 73).** As mentioned in the “Materials and Methods,” conjugation was observed only once in a raw culture. The protargol slides contained many exconjugants but only 15 pairs. The conjugation stages were defined according to the nuclear events.

**Pair formation (Fig. 17, 32).** Only one pair was in a very early stage of pair formation, showing that the partners are much smaller than vegetative cells ( $\sim 105 \times 13$  vs.  $210 \times 20 \mu\text{m}$ ). Further, all conjugating specimens possess distinctly fewer ciliary rows (13 vs. 21), suggesting more than one preconjugation division. Whether conjugation is isogamic or anisogamic is difficult to state due to the few pairs available. Obviously, it is

Table 1. Morphometric data on vegetative (V) and exconjugant (E) specimens of *Dileptus tirjakovae*.

Characteristics <sup>a</sup>	State	Mean	M	SD	SE	CV	Min	Max	n
Body, length	V	212.3	218.0	40.8	8.9	19.2	148.0	281.0	21
	E	87.8	78.0	31.8	5.0	36.2	40.0	153.0	41
Body, width	V	20.4	20.0	4.2	0.9	20.8	15.0	31.0	21
	E	15.0	15.0	2.8	0.4	18.5	10.0	22.0	41
Body length:width, ratio	V	10.6	9.9	2.2	0.5	20.7	7.5	15.0	21
	E	5.9	5.5	2.1	0.3	35.1	2.7	10.6	41
Anterior body end to oral opening, length	V	86.4	86.0	17.3	3.8	20.0	51.0	115.0	21
	E	28.1	20.0	18.8	2.9	66.9	2.0	66.0	41
Proboscis, % of body length	V	40.8	41.1	4.6	1.0	11.2	34.5	56.1	21
	E	29.4	30.5	11.8	1.8	40.0	2.8	50.8	41
Oral opening, length <sup>b</sup>	V	12.3	12.0	1.8	0.4	14.6	10.0	16.0	21
	E	6.8	7.0	1.6	0.2	23.1	4.0	10.0	41
Oral opening, width <sup>b</sup>	V	9.7	9.0	2.4	0.8	25.2	7.0	14.0	10
	E	5.6	6.0	1.3	0.3	23.4	3.0	7.0	15
Oral basket, maximum length	V	23.4	23.0	3.8	0.8	16.3	18.0	32.0	21
	E	15.5	16.0	4.1	0.7	26.8	6.0	23.0	40
Anterior body end to macronucleus, distance	V	119.8	116.0	25.2	5.5	21.0	70.0	159.0	21
Nuclear figure, length	V	19.2	20.0	3.6	0.8	18.8	14.0	31.0	21
	E	15.6	16.0	2.8	0.4	18.2	10.0	22.0	41
Anterior macronuclear nodule, length	V	11.9	12.0	2.3	0.5	19.7	9.0	16.0	21
Anterior macronuclear nodule, width	V	11.9	12.0	1.9	0.4	15.9	8.0	15.0	21
Posterior macronuclear nodule, length	V	11.5	10.0	2.1	0.5	18.5	9.0	16.0	21
Posterior macronuclear nodule, width	V	11.5	12.0	1.7	0.4	15.2	8.0	15.0	21
Macronuclear nodules, number	V	2.0	2.0	0.0	0.0	0.0	2.0	2.0	21
	E	2.0	2.0	0.4	0.1	19.7	1.0	3.0	41
Micronucleus, largest diameter	V	2.7	2.5	—	—	—	2.5	3.0	16
Micronucleus, number	V	1.0	1.0	0.0	0.0	0.0	1.0	1.0	16
Ciliary rows, number	V	21.3	21.0	2.5	0.5	11.8	19.0	29.0	21
	E	13.8	14.0	2.4	0.4	17.3	9.0	19.0	41
Cilia in mid-body in 10 µm, number	V	4.7	5.0	0.6	0.1	12.4	4.0	6.0	21
Anterior body end to last dorsal brush dikinetid, distance	V	49.3	50.0	10.5	2.3	21.3	35.0	72.0	21
	E	16.1	14.0	12.1	1.9	75.4	0.0	43.0	41
Dorsal brush dikinetids, total number	V	39.5	40.0	8.6	1.9	21.8	22.0	59.0	21
	E	11.9	8.0	10.0	1.6	83.5	0.0	32.0	41
Dikinetidal portion of dorsal brush, % of body length	V	23.4	23.9	3.2	0.7	13.5	18.1	27.8	21
Groups of excretory pores, number	V	6.3	6.0	1.2	0.4	18.4	5.0	8.0	10

<sup>a</sup>Data based on mounted, protargol-impregnated, and randomly selected specimens from a non-flooded Petri dish culture. Measurements in µm. CV, coefficient of variation in %; M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of specimens investigated; SD, standard deviation; SE, standard error of mean.

<sup>b</sup>Measured as distance between circumoral kinety.

isogamic with respect to shape and morphology, while probably anisogamic with respect to size because the average length ratio of the partners is 1.4:1 in the 15 pairs measured.

Pair formation is heteropolar, namely, the partners unite with the ventral side in such a way that the top of one proboscis is placed on the base of the other (Fig. 17, 32). The right side of the unit area of one partner faces the right side of the unit area of the other partner: thus the perioral kineties and the right branch of the circumoral kinety are visible when the specimens are observed in the same focal plane (Fig. 32, 35). The union mode is ventral-to-

dorsal in terms of the dorsal brush because the brush of only one partner is visible when the pair is observed in the same focal plane (Fig. 33, 34). The conjugants may form rod-like or strongly arched pairs; no correlation between pair shape and progress through conjugation was found.

The onset of conjugation is associated with distinct body changes (Fig. 17): (1) the proboscis shortens by about 87% causing the cells first to become spatulate and then more or less fusiform; (2) both the internal and external oral baskets lose integrity and become smaller; and (3) the number of contractile vacuoles

Table 2. Comparison of macronuclear pattern in vegetative and exconjugant specimens of *Dileptus tirjakovae*.

State	Macronuclear pattern (proportion, %)					Number of specimens analyzed
	Two globular nodules	Two ellipsoidal nodules	Four globular nodules	One globular nodule	One ellipsoidal nodule	
Vegetative cells	98.8	—	—	0.4	0.8	866
Early exconjugants <sup>a</sup>	83.3	6.5	1.3	2.8	6.1	582
Late exconjugants <sup>b</sup>	89.6	—	2.6	2.6	5.2	77

<sup>a</sup>Exconjugants with few food vacuoles.

<sup>b</sup>Exconjugants with several fresh food vacuoles.

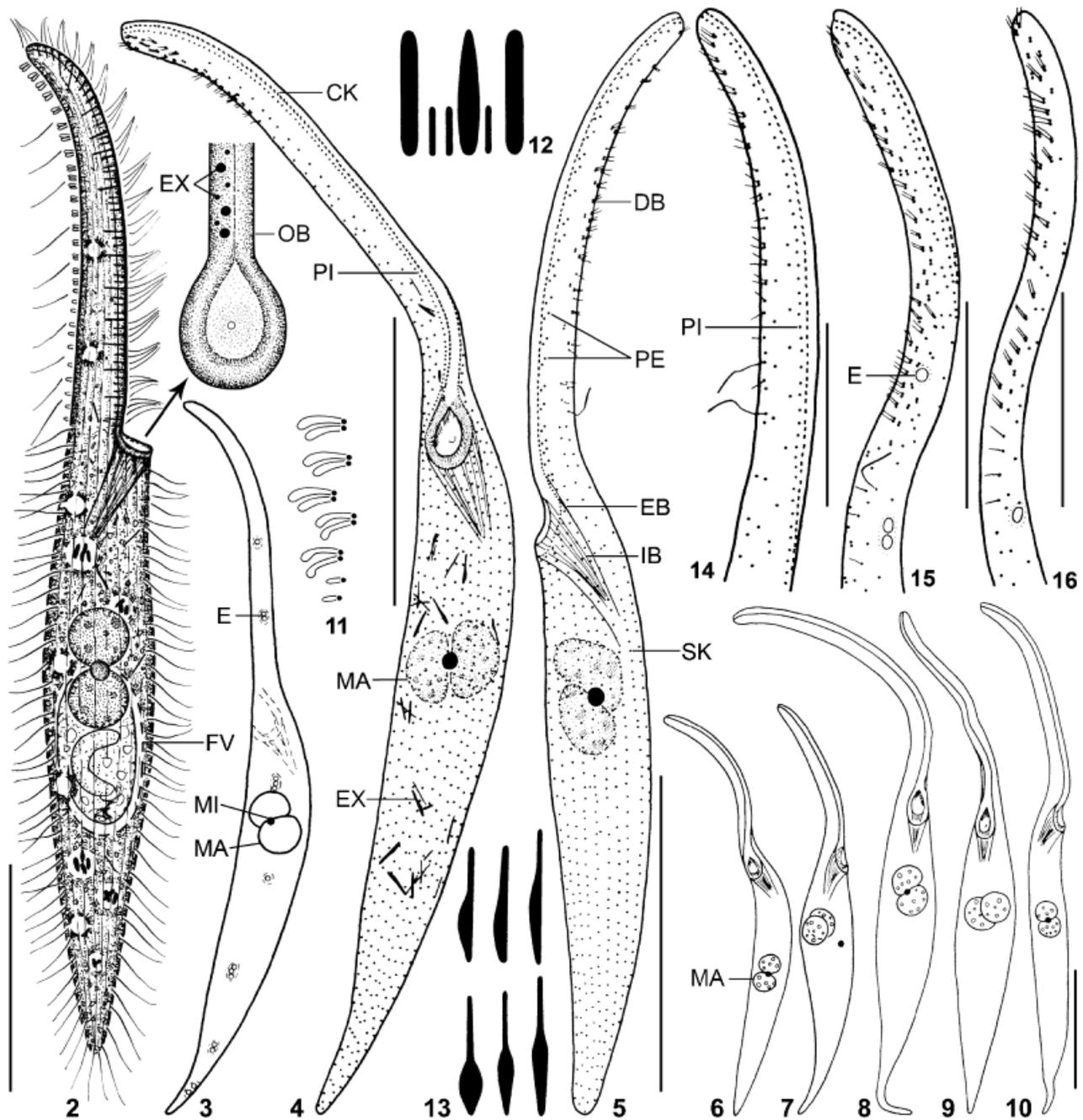


Fig. 2–16. *Dileptus tirjakovae* n. sp. from life (2, 11–13) and after protargol impregnation (3–10, 14–16). 2. Right side view of a representative specimen with oral area in frontal view. Note the two types of extrusomes attached to only the broader right bulge half. 3. Excretory pore and nuclear pattern. 4, 5. Ciliary pattern of ventral and left side and nuclear apparatus of holotype and paratype specimen. 6–10. Variability of body shape and size as well as of nuclear apparatus. Drawn to scale. 11. Part of dorsal brush. 12. Two types of basically rod-shaped oral bulge extrusomes, type I about 6  $\mu\text{m}$ , type II 2.5–3  $\mu\text{m}$  long. 13. Partially exploded type I extrusomes. 14–16. Dorsal and dorsolateral views of ciliary pattern of proboscis. Figure 14 shows the right side of Fig. 5. Brush dikinetids are scattered on dorsal surface; brush bristles lacking or not impregnated in some dikinetids. CK, circumoral kinety; DB, dorsal brush; E, excretory pore(s) of a contractile vacuole; EB, external basket; EX, extrusomes; FV, food vacuole; IB, internal basket; MA, macronucleus; MI, micronucleus; OB, oral bulge; PE, preoral kineties; PI, perioral kinety; SK, somatic kinety. Scale bars = 50  $\mu\text{m}$  (Fig. 2, 4–10) and 20  $\mu\text{m}$  (Fig. 14–16).

decreases. Conspicuous ciliary changes occur in connection with body diminution (Fig. 32): (1) the number of preoral kineties as well as perioral and dorsal brush kinetids distinctly decreases; (2) the anterior portion of the right side somatic kineties begins to curve dorsally; and (3) the interkinetal distances increase. Thus,

early conjugants achieve a spathidiid appearance in body shape and ciliary pattern.

In very early conjugants, the vegetative macronuclear nodules lose their globular shape and commence to fuse and to stretch. Furthermore, the micronucleus swells and shows fibrous



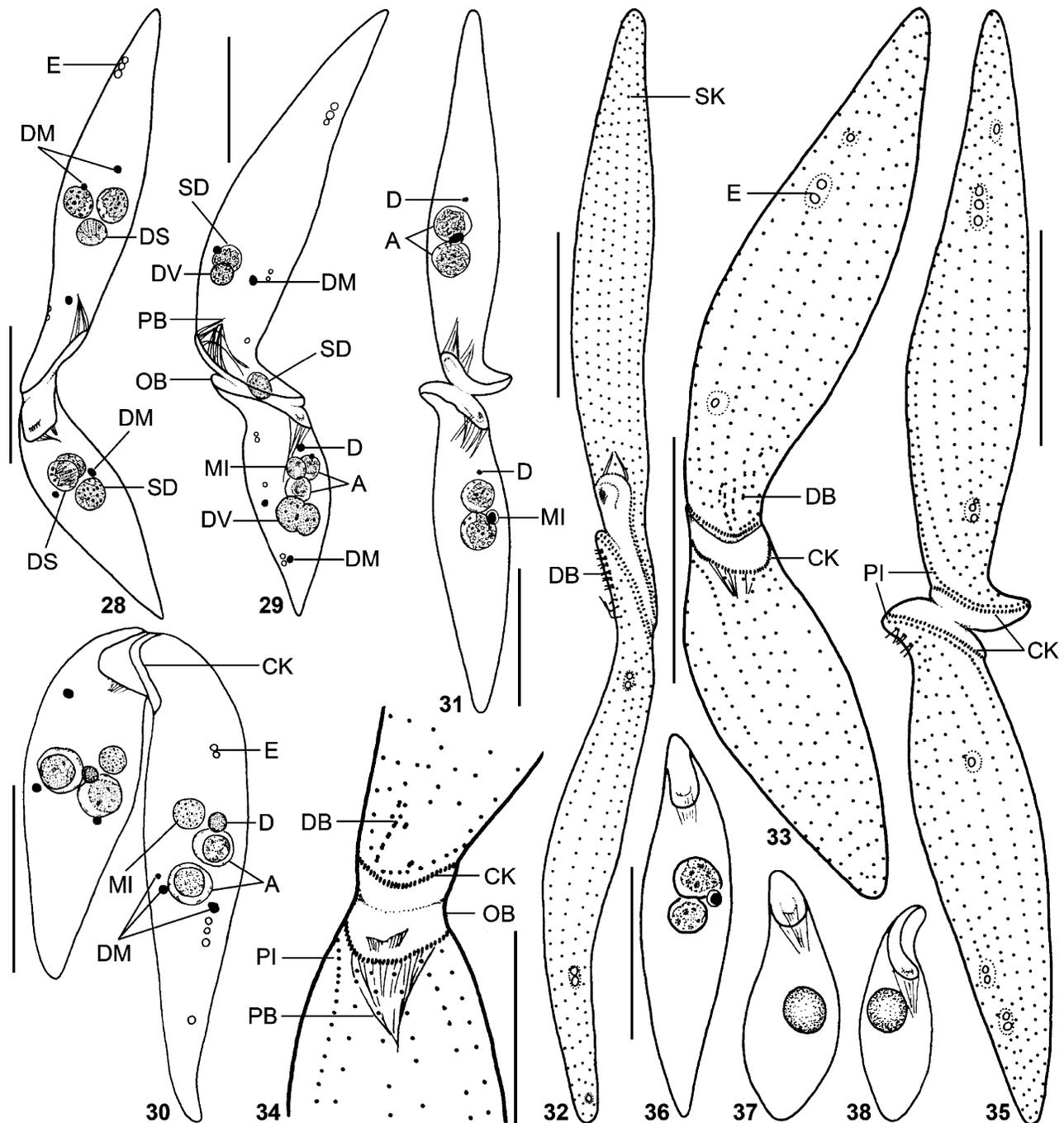


Fig. 28–38. *Dileptus tirjakovae* n. sp., protargol-impregnated conjugants (28–35) and exconjugants (36–38). 28. Prophase of second synkaryon division. 29. Derivatives of the first synkaryon division in the longer partner and derivatives of the second synkaryon division in the shorter partner. 30. Derivatives of second synkaryon division. 31. Very late stage. 32–35. Ciliary pattern of the very early conjugants (32), the mid-conjugants (33, 34), and the very late conjugants (35) shown in Fig. 17, 19, 22, and 31. 36–38. Very early exconjugants, as shown by the small and stout body. Drawn to scale. A, macronuclear anlagen; CK, circumoral kinety; D, degenerating synkaryon derivative; DB, dorsal brush; DM, degenerating maturation derivative; DS, dividing synkaryon derivative; DV, degenerating vegetative macronucleus; E, excretory pores of a contractile vacuole; MI, micronucleus; OB, oral bulge; PB, pharyngeal basket; PI, perioral kinety; SD, synkaryon derivatives; SK, somatic kinety. Scale bars = 30  $\mu$ m.

structures, possibly chromosomes (Fig. 17). The food vacuoles disappear, making cells more transparent.

**Maturation divisions and pronuclei** (Fig. 18–20, 33, 73). When the maturation divisions commence, further body diminution occurs (i.e. from about  $105 \times 13 \mu\text{m}$  to about  $60 \times 17 \mu\text{m}$ ) and conspicuous changes in the length:width ratio

occur, both as compared with the vegetative cells (about 3.6:1 vs. 10.6:1) and the partners (2.9:1 in the shorter vs. 4.2:1 in the longer). The proboscis is reduced to a rounded triangular lip, a massive change associated with conspicuous changes in the oral ciliary pattern (Fig. 33): (1) the circumoral kinety, which is restricted to the ventral side in the vegetative cells, extends now also

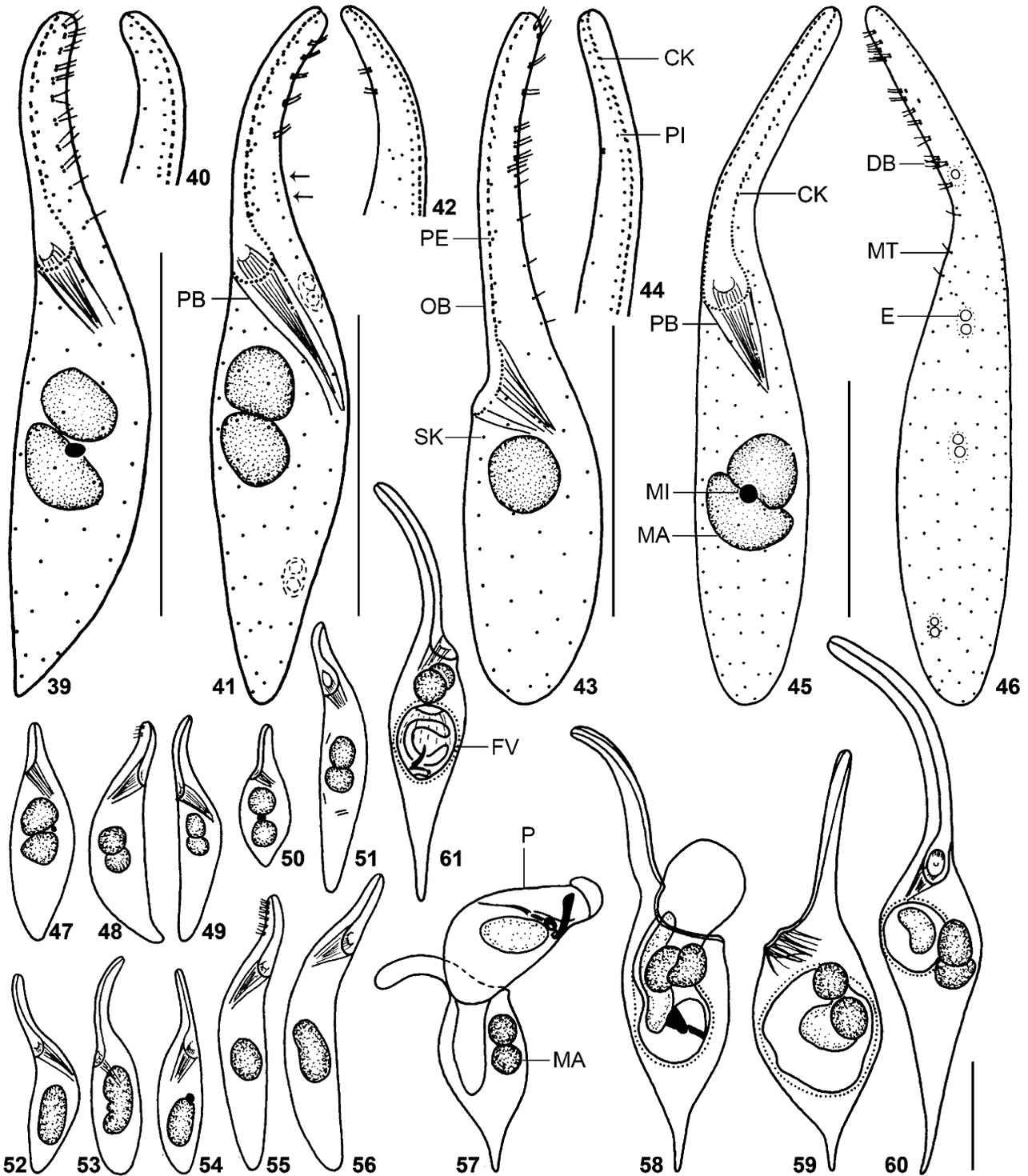


Fig. 39–61. *Dileptus tirjakovae* n. sp., protargol-impregnated exconjugants. 39, 41, 43. Ciliary pattern of left side and nuclear apparatus of early exconjugants. The preoral kinetids appear close to the oral dikinetids. Arrows mark in Fig. 41 non-ciliated brush kinetids. 40, 42, 44. Right-side ciliary pattern of the proboscis of the specimens shown in Fig. 39, 41, and 43. 45, 46. Ciliary pattern of ventral and dorsal side and nuclear apparatus of an early exconjugant. 47–56. Early exconjugants with short proboscis. Macronuclear anlagen may fuse together forming a globular or ellipsoidal mass. 57–60. Exconjugants engulfing *Metopus hasei*. 61. Exconjugant having engulfed a peritrich ciliate. CK, circumoral kinety; DB, dorsal brush; E, excretory pores of a contractile vacuole; FV, food vacuole; MA, macronucleus; MI, micronucleus; MT, monokinetidal tail of dorsal brush; OB, oral bulge; P, prey; PB, pharyngeal basket; PE, preoral kinety; PI, perioral kinety; SK, somatic kinety. Scale bars = 30  $\mu$ m; Fig. 47–61 drawn to scale.

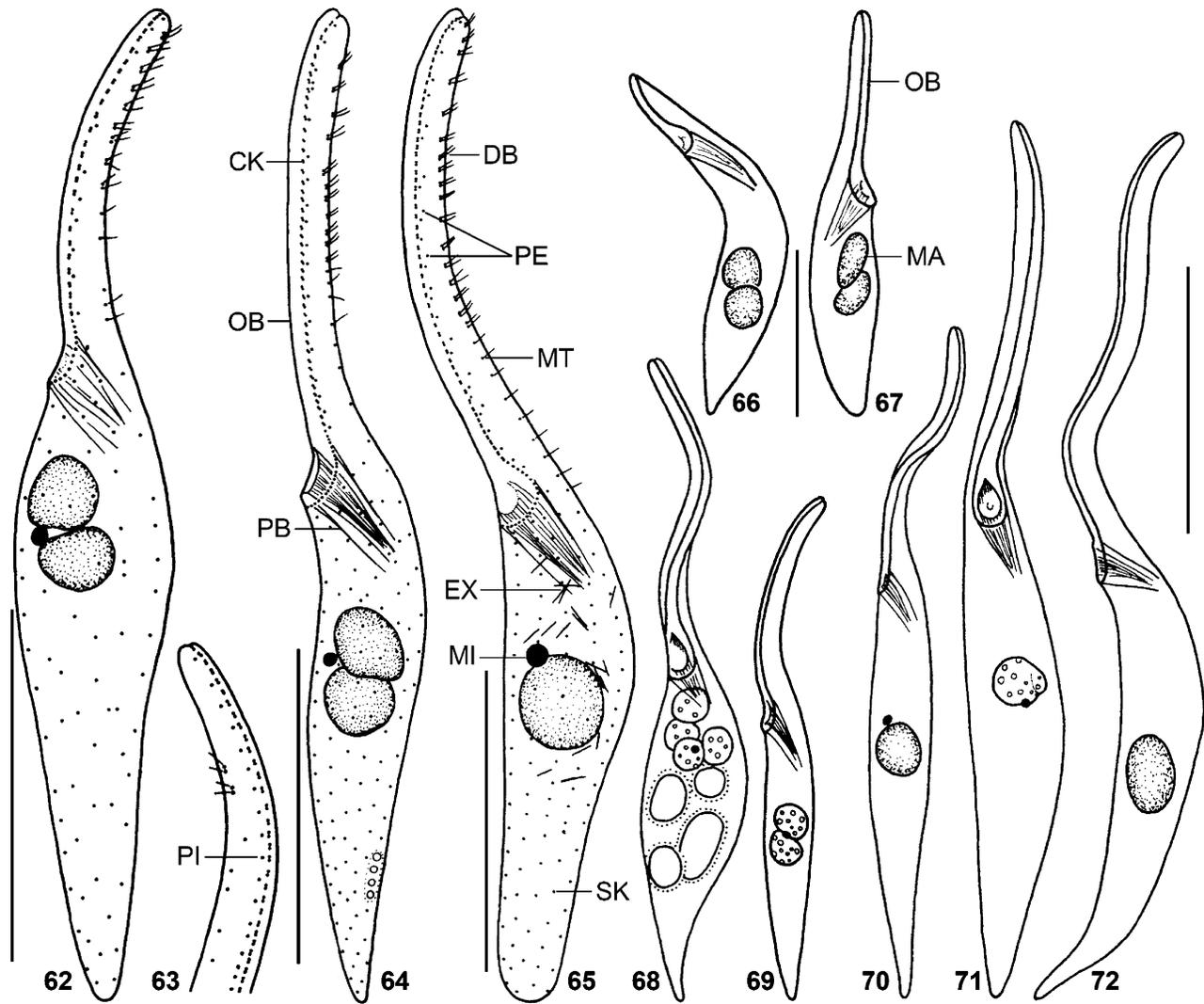


Fig. 62–72. *Dileptus tirjakovae* n. sp., protargol-impregnated exconjugants. 62, 64. Ciliary pattern of left side and nuclear apparatus of late exconjugants. Note that the preoral kinetids form a very loose kinety resembling the right side perioral kinety. 63. Right side ciliary pattern of the proboscis of the specimen shown in Fig. 62. 65. Ventrolateral view of ciliary pattern and nuclear apparatus of a late exconjugant. The definite body shape is regained but body size is still only half of that in vegetative cells. 66, 67. Early exconjugants. Drawn to scale. 68–72. Exconjugant nuclear reconstruction. Macronuclear anlagen may fuse together to an ellipsoidal mass or undergo further division yielding four macronuclear anlagen. Drawn to scale. CK, circumoral kinety; DB, dorsal brush; EX, extrusomes; MA, macronucleus; MI, micronucleus; MT, monokinetid tail of dorsal brush; OB, oral bulge; PB, pharyngeal basket; PE, preoral kineties; PI, perioral kinety; SK, somatic kinety. Scale bars = 30  $\mu$ m (Fig. 62–67) and 50  $\mu$ m (Fig. 68–72).

along the dorsal side, and the oral dikinetids possibly assume a more vertical orientation; (2) there is a further decrease in the number of perioral kinetids; and (3) the preoral kineties may even be entirely resorbed. Eventually, the shape of maturing specimens becomes *Enchelyodon*- or *Protospathidium*-like (i.e. resembles “polar” haptorids without proboscis; Fig. 18, 19, 33).

There are three maturation divisions. During the first division, the micronucleus swells from a diameter of about 2.7 to 7–8  $\mu$ m (Fig. 17). The second division yields four globular maturation derivatives that impregnate homogeneously and slowly degenerate, except for one which enters the third maturation division (Fig. 18, arrows). The degenerating maturation derivatives impregnate more heavily than the disintegrating macronucleus (Fig. 20). The remaining maturation derivative moves into the anterior body end and assumes a highly characteristic, fusiform shape during prophase (Fig. 18). Then the derivative begins to divide producing a conspicuous

spindle (Fig. 19), which is resorbed before the synkaryon develops (Fig. 20). One pronucleus becomes stationary, while the other migrates into the partner to form the synkaryon (Fig. 20, 21, 73).

*Synkaryon formation and synkaryon divisions* (Fig. 21–30, 34, 73). No further changes occur in body shape and ciliary pattern during synkaryon formation and the first synkaryon division (Fig. 34). After the first synkaryon division, the oral area commences to elongate to a spathidiid oral bulge (Fig. 25, 26, 28, 29). This is associated with conspicuous changes in the ciliary pattern (Fig. 35): (1) the circumoral kinety arranges around the ventral portion of the emerging proboscis; (2) the number of perioral kinetids increases at the base of the proboscis; and (3) the anterior portion of the right side somatic kineties starts to curve dorsally, highly resembling a spathidiid ciliary pattern. Thus, the enchelyodontid pattern, characteristic for the maturing specimens, becomes spatulate.

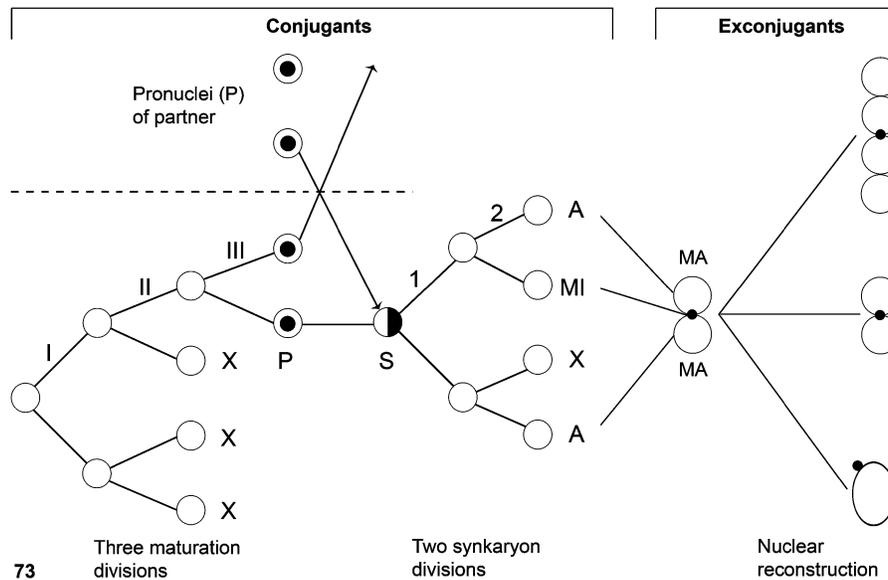


Fig. 73. Scheme of the nuclear processes during and after conjugation of *Dileptus tirjakovae* n. sp. There are three maturation divisions and two synkaryon divisions, which produce four synkaryon derivatives, of which two become macronuclear anlagen, one becomes the micronucleus, and one degenerates. The majority of exconjugants (83%) have the vegetative macronuclear pattern: two globular macronuclear nodules; rarely do the macronuclear nodules fuse to a globular or ellipsoidal mass or undergo a further division producing four globular nodules. I, II, III, maturation divisions; 1, 2, synkaryon divisions; A, macronuclear anlagen; MA, macronucleus; MI, micronucleus; P, pronuclei; S, synkaryon. In the conjugants and exconjugants, circles are nuclear derivatives of divisions. Crosses mark degenerating nuclei.

A synkaryon each is formed in the partners by fusion of the migratory pronucleus with the stationary one (Fig. 21). The first synkaryon division follows pronuclear fusion and is characterized by an inflation of the synkaryon and the appearance of at least 10 chromosomes attached to fibrous structures during metaphase (Fig. 22, 23). The first synkaryon division generates two globular, abutting synkaryon derivatives (Fig. 24, 25), which stepwise divide mitotically (Fig. 26–28), producing four synkaryon derivatives in each partner (Fig. 30). The individual synkaryon derivatives are about 7  $\mu\text{m}$  across and are covered by a distinct membrane slightly separated from the nucleoplasm, which contains many faintly impregnated granules. Finally, two synkaryon derivatives become macronuclear anlagen; one differentiates into the micronucleus; and the last degenerates (Fig. 29, 30). The degenerating vegetative macronucleus and the maturation derivatives are still recognizable in some late conjugants (Fig. 27, 29, 73).

**Pair separation (Fig. 31, 35).** Slightly before separation, the partners form a rod-like structure connected with the basal portion of the proboscides (Fig. 31). Conjugants separate in the spathidiid stage after the second synkaryon division, when the nuclear apparatus consists of two macronuclear anlagen and one micronucleus in the vertex formed by these abutting anlagen. The resorption of the vegetative macronucleus and maturation derivatives is now complete because they are absent from very late conjugants and early exconjugants (Fig. 31).

**Exconjugant reorganization (Tables 1 and 2 and Fig. 36–73).** **Cell reorganization.** In the absence of detailed data from cultures, we could not distinguish with certainty between exconjugants and preconjugants. However, we could distinguish early and mid-exconjugants and/or preconjugants from vegetative specimens by their much smaller size ( $88 \times 15$  vs.  $212 \times 20$   $\mu\text{m}$ ), the stouter body ( $\sim 6:1$  vs.  $10.5:1$ ), and by the much shorter proboscis ( $28$  vs.  $86$   $\mu\text{m}$ ). Further, there were only 15 conjugation pairs among over 500 small and very small supposed exconjugants, suggesting that we missed the peak of conjugation and thus also preconjugation division(s).

Postconjugational reorganization is associated with intense proboscis and body growth (Fig. 39–46, 62–72), as shown by body length:width ratio ( $6:1$  in exconjugants vs.  $\sim 4:1$  in conjugating specimens). The reconstruction of the oral basket begins in late conjugants (Fig. 25, 28, 29, 31) and is completed soon after pair separation (Fig. 37–39, 41, 45). Thus, the basket is relatively larger in the small exconjugants than in the large vegetative cells (Table 1 and Fig. 41, 52–56). The number of groups of excretory pores increases and they soon appear in the growing proboscis (compare Fig. 41 with Fig. 46). Many developing extrusomes, which impregnate heavily with protargol, are scattered in the cytoplasm (Fig. 51, 65) and possibly migrate into the proboscis where they lose impregnability (see description of species). Subsequently, the exconjugants feed on medium-sized ciliates to gather nutrients for further growth and reorganization (Fig. 57–61). The definitive body shape is eventually regained in late exconjugants (Fig. 64, 65, 69–72), while reaching the vegetative body size needs one or several postconjugation divisions.

**Ciliary pattern.** The general appearance and ciliary pattern of very early exconjugants resembles that of *Spathidium sensu lato* due to the short, only slightly projecting proboscis. The very early exconjugants have widely spaced somatic kinetids (Fig. 39, 41, 43, 62), which proliferate throughout the kineties causing late exconjugants to be ordinarily ciliated (Fig. 64, 65). The number of ciliary rows remains unchanged in conjugants and exconjugants (on average 13 and 14, respectively), showing that the vegetative number (on average 21) is regained during or after postconjugation division(s).

In very early exconjugants, the dorsal brush is rather inconspicuous and composed of only a few dikinetids (Fig. 48, 55). However, non-ciliated basal bodies or dikinetids do appear near the posterior end of the parental remnants of the dorsal brush (Fig. 41, arrows). They originate from either restructured somatic kinetosomes or, more likely, de novo because almost the entire dorsal surface of the proboscis is covered with paired and single brush bristles in late exconjugants (Fig. 64, 65); however, their number is still much lower than in vegetative cells (Table 1).

Table 3. Comparison of main conjugation events in three *Dileptus* species.

Characteristics	<i>Dileptus tirjakovae</i> (present study)	<i>Dileptus anser</i> (Vinnikova 1974a)	<i>Dileptus gigas</i> <sup>a</sup> (Visscher 1927)
Early conjugants, length	Distinctly shorter than vegetative cells (105 µm vs. 210 µm)	Slightly shorter than vegetative cells (130 µm vs. 160 µm) <sup>b</sup>	Distinctly shorter than vegetative cells (175 µm vs. 400 µm)
Early conjugants, number of ciliary rows	Less than vegetative cells (13 vs. 21)	Not known	Not known
Conjugation division	Probably no	May occur	No
Type of conjugation	Heteropolar and temporary	Heteropolar and temporary	Heteropolar and temporary
Union mode	Dorsal-to-ventral	Dorsal-to-ventral	Dorsal-to-ventral
Body becomes shorter and stouter, and proboscis shortens immensely	Yes	Yes	Yes
Ciliary changes	Distinct	Distinct <sup>c</sup>	Not known
Micronuclei undergoing maturation divisions, number	1	Almost all	Only one of the many
Maturation divisions, number	3	3	3
Differences between pronuclei	Slightly different in size	Different in size and shape	No differences
Synkaryon divisions, number	2	1–4 (2) <sup>d</sup>	1 (4) <sup>e</sup>
Macronuclear anlagen, number	2	1–11 (4) <sup>d</sup>	1 (4) <sup>e</sup>
New micronuclei, number	1	1–4 (1) <sup>d</sup>	1
Degenerating synkaryon derivatives, number	1	0	0
Pair separation	After formation of macronuclear anlagen	After formation of macronuclear anlagen <sup>f</sup>	After formation of synkaryon

<sup>a</sup>Likely a misidentification. The species probably belongs to the *Dileptus margaritifera* group.

<sup>b</sup>Length of the cells measured without the proboscis.

<sup>c</sup>This is sustained by observations of Golińska and Afon'kin (1993).

<sup>d</sup>Numbers in brackets indicate the most commonly found number.

<sup>e</sup>Numbers in brackets are according to Hertwig (1904).

<sup>f</sup>Conjugants separate usually about 24 h after the onset of conjugation.

The perioral kinety is preserved during conjugation but in a rather reduced state (Fig. 35). During growth of the proboscis, the perioral kinety elongates by intrakinetal proliferation of basal bodies until it attains the vegetative appearance (Fig. 44, 63). The oblique preoral kineties, which are usually composed of two basal bodies, are entirely resorbed during conjugation (Fig. 33, 34). Soon after pair separation some monokinetids appear close to the left side oral dikinetids, forming a very loose kinety resembling the right side perioral kinety (Fig. 39, 41, 43, 45, 62). Later, the number of preoral basal bodies increases concomitantly with proboscis elongation (Fig. 64), but the vegetative pattern is obtained only in late exconjugants (Fig. 65).

**Nuclear apparatus.** Among 582 early exconjugants, about 83% have the vegetative macronuclear pattern (i.e. two globular nodules; Fig. 36, 39, 41, 47–51, 66), 6.5% possess two ellipsoidal nodules (Fig. 67), 6% have one ellipsoidal nodule (Fig. 52–54, 56), 3% have one globular nodule (Fig. 37, 38, 43, 55), and only 1.5% have four nodules (Table 2). Thus, the macronuclear anlagen may fuse to a globular or ellipsoidal mass or may occasionally undergo further division as is shown by exconjugants with four globular nodules (Fig. 68, 73). The micronucleus is slightly larger in very early exconjugants than in late ones, and is surrounded by a membrane, which is visible only soon after the separation of the conjugants, i.e. in specimens with very short proboscis (Fig. 31, 36).

## DISCUSSION

**Classification of *Dileptus tirjakovae* n. sp.** Jankowski (1967) split *Dileptus* into three genera solely based on the macronuclear configuration: *Dileptus* with dispersed nodules; *Dimacrocarion* with two nodules and a single micronucleus in between; and *Monilicaryon* with moniliform macronucleus. Foissner's (1997) re-investigation of *Dileptus monilatus*, type of *Monilicaryon*, showed that the same nuclear pattern evolved independently in

several evolutionary lines of *Dileptus* sensu lato. Thus, we classify our species traditionally into the genus *Dileptus* until further, especially, molecular data suggest differently.

Kahl (1931) and Dragesco (1963), the last revisers of *Dileptus*, used the following features for distinguishing species within the genus: body shape and size, ratio of body and proboscis length, the nuclear and contractile vacuole pattern, and details of the dorsal brush. Later, Foissner (1984, 1989) and Foissner et al. (2002) added extrusome shape and various morphometrics. Using these features, *D. tirjakovae* n. sp. is almost unique in having two abutting, globular macronuclear nodules with a micronucleus in the vertex of the nodules, a highly constant pattern found in over 800 specimens. Although a similar pattern is found in many species (e.g. Dragesco 1963; Foissner 1989; Kahl 1931), the nodules are globular only in *D. tirjakovae* and *Dileptus lacazei*, while oblong, bluntly clavate, or reniform in all other species. Thus, we use the nuclear pattern as a main feature for separating *D. tirjakovae* from all other described *Dileptus* species, except for *D. lacazei* which has the same pattern and also lives in saline environments. However, *D. lacazei* has a distinct tail and a highly contractile proboscis, two features emphasized in the original description (Gourret and Roeser 1886) and the authoritative redescription by Dragesco (1963), while both features are not properties of *D. tirjakovae*.

A further feature possibly characterizing *D. tirjakovae* is the dorsal brush, composed of scattered kinetids, a pattern as yet found only in *D. anser*, a much larger ciliate with moniliform macronucleus and very long proboscis (Wirnsberger, Foissner, and Adam 1984). All other dileptids impregnated so far (about 15 species) have staggered brush rows with fairly ordered kinetids.

**Conjugation.** Conjugation of *D. tirjakovae* basically agrees with data from congeners *D. anser* and *Dileptus cygnus* (Golińska and Afon'kin 1993; Vinnikova 1974a, 1976; Visscher 1927). Differences occur in the number of micronuclei undergoing maturation, in the number of synkaryon divisions, in the number of

macronuclear anlagen, in the number of degenerating synkaryon derivatives, and in pair separation with respect to nuclear development (Table 3).

*Dileptus* offers an example of a haptorid ciliate with a proboscis at the base of which is the oral opening. Despite this curious body organization, *Dileptus* conjugation is consistent with some previous observations on didiniid, acaryophryid, and spathidiid haptorids (Prandtl 1906; Serrano, Martín-González, and Fernández-Galiano 1990; Xu and Foissner 2004) in that it is temporary, heteropolar, and partners unite bulge-to-bulge. However, some peculiarities occur and may be even unique to dileptids: the massive changes in body shape and ciliary pattern, causing early and late conjugants to resemble *Spathidium* (Fig. 32, 35), while mid-conjugants resemble *Enchelyodon* or *Protospathidium* (Fig. 33, 34). These and molecular data (Strüder-Kypke et al. 2006) give some support to Xu and Foissner (2005), who speculated that spathidiids evolved from a *Dileptus*-like ancestor by reduction of the proboscis. Furthermore, some spathidiids have a dorsal elongation of the oral bulge (Foissner and Xu 2007), which could be a proboscis vestige.

To date, heteropolar, temporary conjugation has been reported only in litostome ciliates, for instance, in *Acaryophrya collaris*, *Didinium nasutum*, several dileptids, and some spathidiids (Golińska and Afon'kin 1993; Prandtl 1906; Serrano et al. 1990; Vinnikova 1974a, 1976; Visscher 1927; Xu and Foissner 2004), as well as in the endosymbiotic entodiniomorphids (Raikov 1972). Trachelophyllids and pleurostomatids, two other common haptorid groups, form homopolar pairs: pleurostomatids conjugate in a unique way in that the oral area of one partner faces the dorsal side of the other partner (Kahl 1931; Prowazek 1909), while trachelophyllids unite in mid-body (Bürger 1906). Thus, it seems that the conjugation mode is not related to mouth location. Further, the union mode is at best specific to family or order level.

**Exconjugants and cell reorganization.** Previous investigations focused on the nuclear events (Prandtl 1906; Vinnikova 1974a; Visscher 1927). The present study and those of Serrano et al. (1990) and Xu and Foissner (2004) are the only ones that investigated concomitantly reorganization of cell shape, nuclear apparatus, and ciliary pattern, but they have the same failure: pre- and postconjugation divisions were not studied.

Confirming previous data, early exconjugants of *Dileptus* are much smaller and stouter than vegetative cells and may display a deviating nuclear pattern, namely, four globular macronuclear anlagen (Vinnikova 1974a; Visscher 1927). The exconjugants need several days to complete nuclear reorganization and to achieve the definite body shape and size. For instance, nuclear reorganization in *D. gigas* takes 3–4 days and the vegetative nuclear pattern is regained only after exconjugation division, which occurs later than 4 days after conjugation (Visscher 1927). Likewise, one or several postconjugation divisions must occur in *D. tirjakovae* because the exconjugants are much smaller and have fewer ciliary rows than vegetative cells. Indeed, such specimens might be easily mistaken for a distinct species, and thus the exconjugation data are of high significance for  $\alpha$ -taxonomy in general.

Unlike spathidiids (Xu and Foissner 2004), dileptids display conspicuous changes in body shape and ciliary pattern before, during, and after conjugation (this study; Golińska and Afon'kin 1993; Visscher 1927). The reconstruction of the proboscis and its infraciliature is the most remarkable process in postconjugational reorganization of *D. tirjakovae*. Possibly, it follows the events known from cells with experimentally removed proboscis and investigated with transmission electron microscopy by Golińska (1978, 1983): (1) new parts of the circumoral and perioral kinety arise as a prolongation of the old ones from a productive region at the base of the proboscis, and (2) ordinary somatic kinetids can transform into brush dikinetids. These processes cannot be proven

unambiguously in the light microscope. However, we observed (1) that the kinetids of the circumoral and perioral kinety are more densely spaced at the base of the proboscis and (2) that the lack of an anlagen field suggests that somatic kinetids may transform into brush dikinetids. Unfortunately, Golińska (1978) did not mention the reconstruction of the preoral kineties. Our data show that the basal bodies of the new preoral kineties occur very near the circumoral kinety, indicating that they may originate from oral dikinetids and then divide once to form a kinety with two cilia.

#### TAXONOMIC SUMMARY

Class Litostomatea Small et Lynn, 1985  
Subclass Haptorina Corliss, 1979  
Order Haptorida Corliss, 1974  
Suborder Dileptina Jankowski, 1978  
Family Tracheliidae Ehrenberg, 1838  
*Dileptus tirjakovae* n. sp.

**Diagnosis.** Size about  $230 \times 25 \mu\text{m}$  in vivo. Shape dileptid with proboscis occupying about 40% of body length. Two globular, abutting macronuclear nodules with a micronucleus in nodule vertex. A dorsal row of contractile vacuoles. Two size-types (6 and  $2.5\text{--}3 \mu\text{m}$ ) of basically rod-shaped extrusomes attached to right half of oral bulge. On average 21 ciliary rows; dorsal brush kinetids scattered on dorsal surface of proboscis. Oral opening roundish, about  $12 \times 9 \mu\text{m}$  in size.

**Type locality.** Highly saline coastal soil from the bay of Nauplia, Greece, E  $22^{\circ}43'N$   $37^{\circ}33'$ .

**Type material.** One holotype slide and six paratype slides with protargol-impregnated specimens have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LL), Austria. Relevant vegetative and conjugating individuals as well as exconjugants are marked by black ink circles on the coverslip.

**Dedication.** Named in honour of Dr. Eva Tirjaková (Comenius University), as a small token of appreciation for guiding the junior author into ciliatology.

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