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Research Note

USE OF THE PYRIDINATED SILVER IMPREGNATION METHOD TO VISUALIZE CILIARY STRUCTURES IN RESISTANT AND TROPHIC FORMS OF PROTOZOA

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Many standard techniques using silver impregnation have been employed to observe the ciliature and infraciliature of ciliates. The clarity in which the most minute details of these structures were revealed varies according to the methodology used. Utilizing the very simple and fast pyridinated silver method (D. Fernández-Galiano, 1976, *Trans. Am. Microsc. Soc., 95:* 557-560) we succeeded in obtaining more information about the morphogenesis of the ciliary organelles and its primordia during excystation of *Gastrostyla steini* (Ciliate, Hypotrichida). Here onward, minute details of these organelles in the trophic form were also analyzed.

Cysts of *G. steinii* were isolated from one week old cultures in which most of the free swimming cells had already dedifferentiated themselves into a resistant form. The resting cysts were placed in syracuse dishes filled with mineral water enclosed in a humid chamber in order to trigger the excystment process, for approximately 4 h at room temperature. The trophic cells used were selected from log-arithimic phase cultures.

The silver impregnation method consists in the sample fixation for a few seconds with pure commercial formaline (3 drops), followed by the add of pure pyridine (10 drops), distilled water (5 ml), 4% peptone (15 drops) and modified Rio-Hortega's ammoniacal silver carbonate solution[1] (0.5 ml). Next, the cell suspension was well mixed, filled up with distilled water (approximately 18 ml) and shaken in a bath of water at a temperature of 65 °C for a few minutes. When a "cognac" yellow hue was obtained, the reaction was interrupted with 0.5% sodium tiossulfate. The liquid part of the mixture was poured out and the cells left on the bottom rinsed exaustively with distilled water. After this, the cells were picked up one by one with the aid of a micropipette in a stereoscopic microscope, mounted to be observed and registered immediately. Reagents were obtained from Merck and Company, Inc., Rahway, N. J.

The analysis of the ventral surface of the *G. steinii* cell (Fig. 1) revealed the existance of an adoral zone comprised by a collection of 37 membranelles. Each membranelle was formed by three almost equally large kinetosome rows and a very short one (Fig. 2). The oral membranes were easily detected lying on the right side of the peristome (Fig. 2). Differing from some published data (J. N. Grim, 1970, *Trans. Am. Microsc. Soc., 89:* 486-497), our samples showed the paroral membrane to be formed by just two rows of cilia arranged in a zig-zag pattern. The endoral membrane contained double cilium rows. Belonging to the fronto-ventral-transverse system, the frontal cirri were constituted by seven parallel kinetosome rows (Fig. 2), the largest one with approximately nine cilia. The two kinetosome rows on the extreme left had one or two fewer cilia. Contrastingly, the marginal cirri were made up of three or four parallel kinetosome rows of approximately the same size (Fig. 3). In full accordance with Grim's report, the majority of individuals in the population have a single right marginal row of cirri despite the occasional occurrence of double rows. The assemblage of the microtubular organelles were easily

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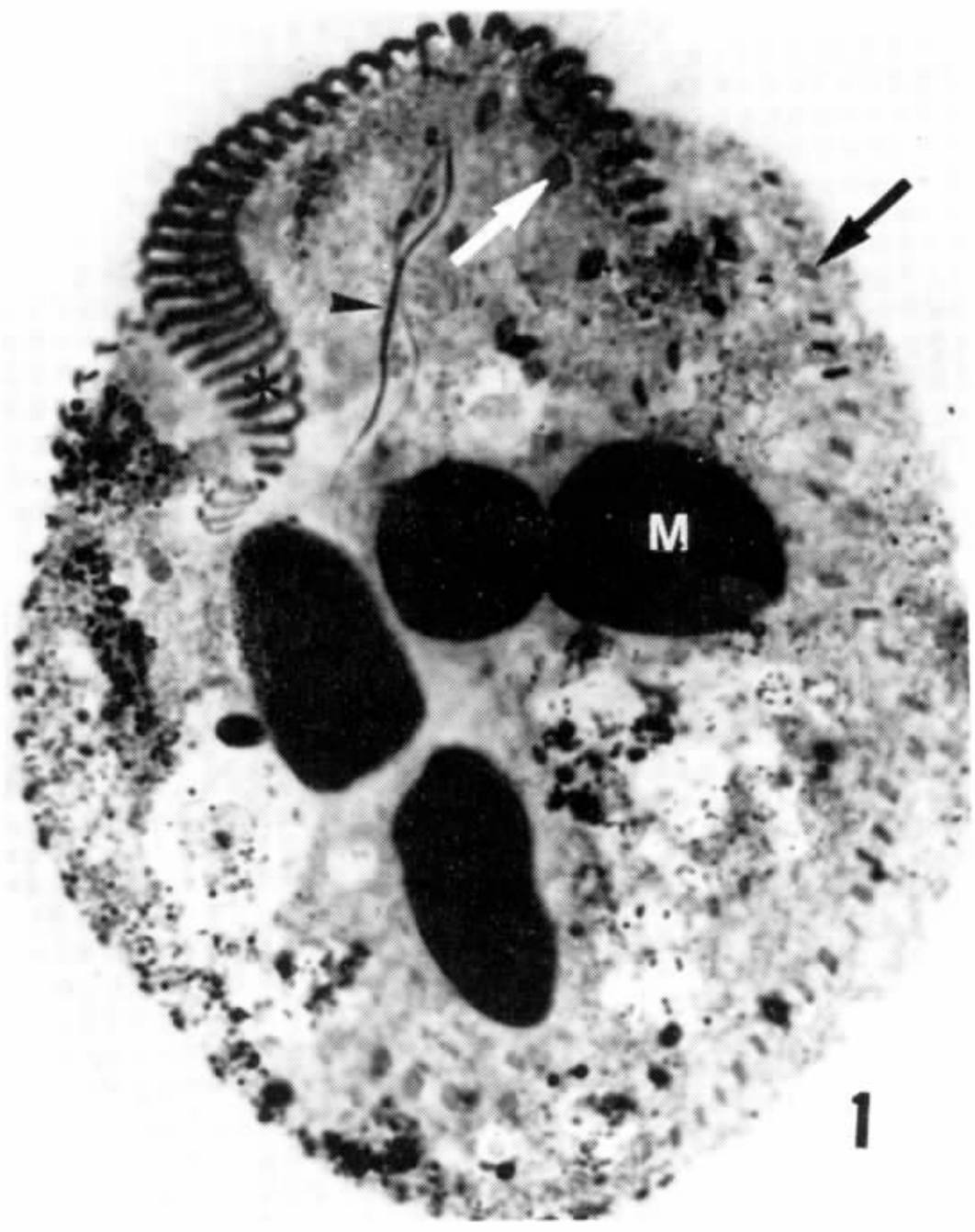
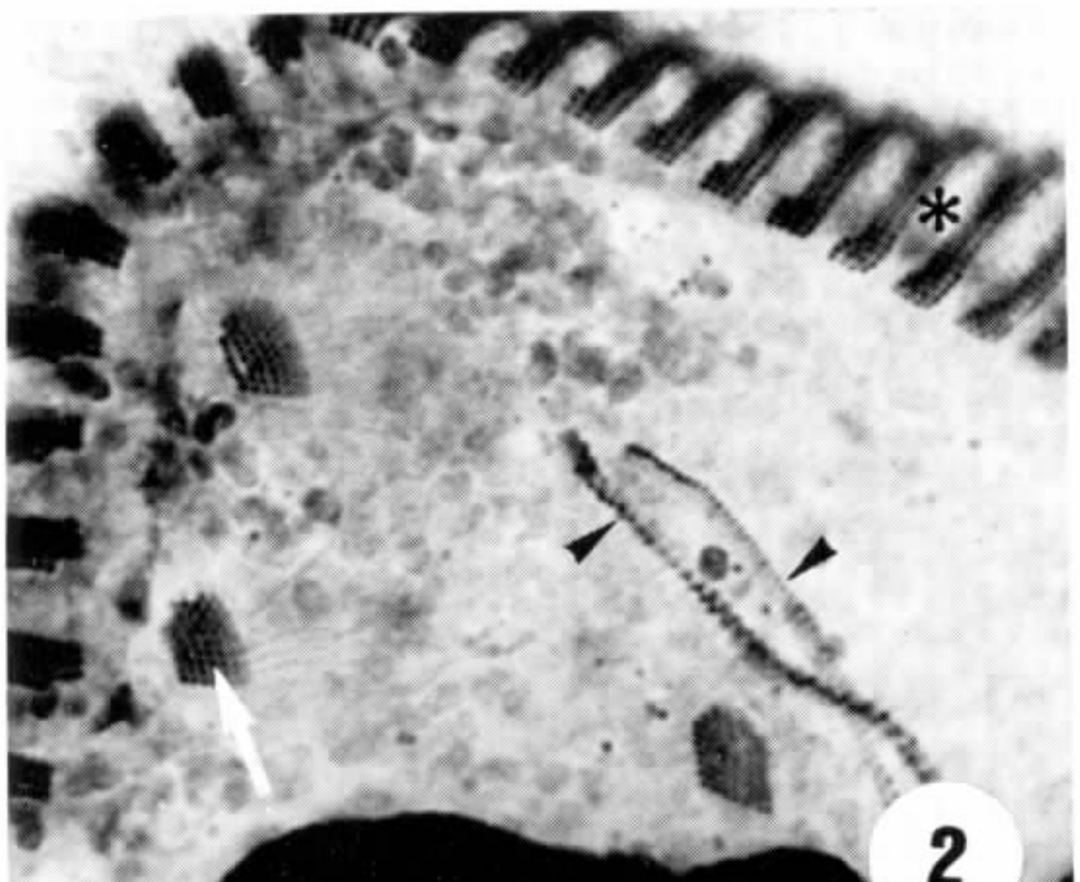
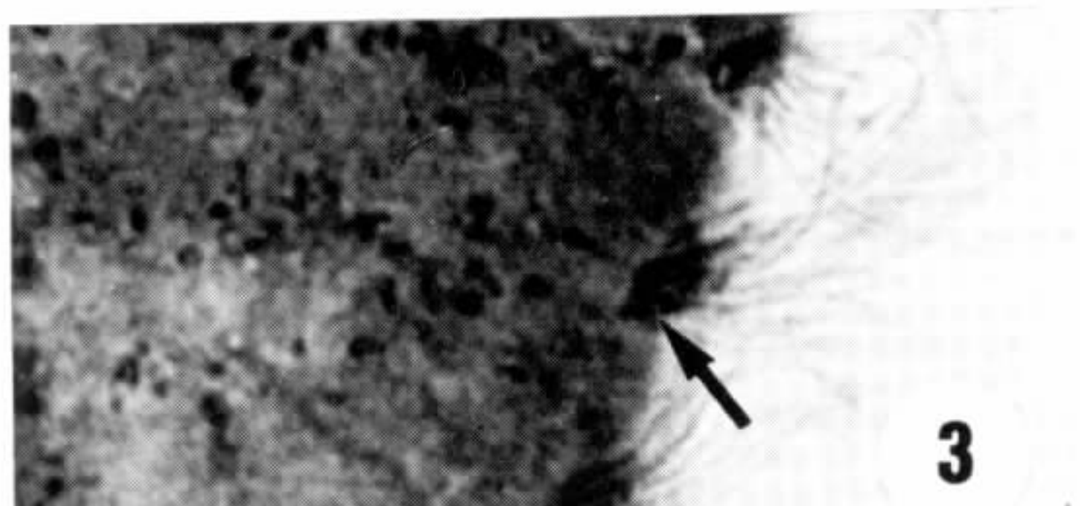
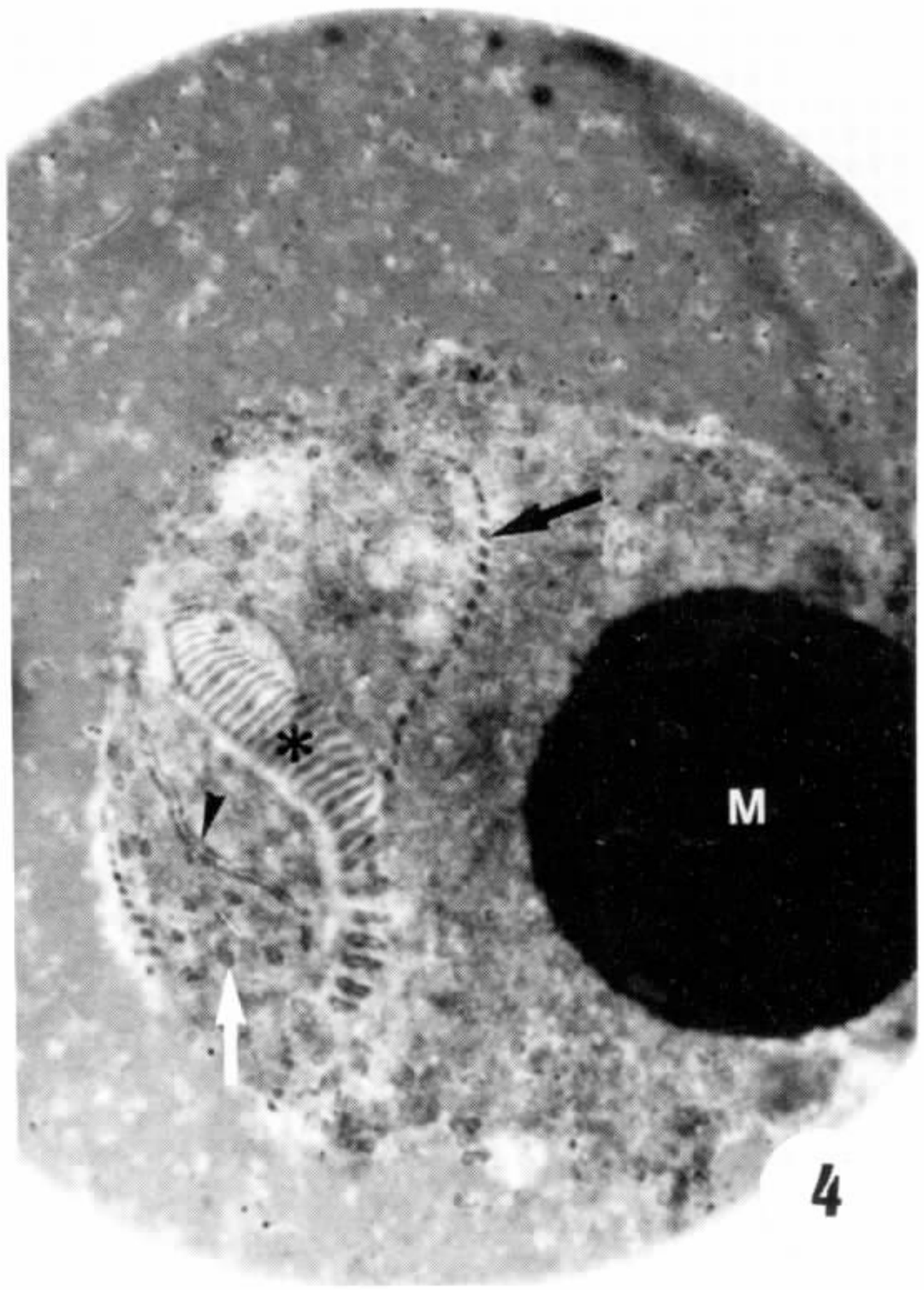
[1] 10% silver nitrate 50 ml
5% sodium carbonate 150 ml
ammonia (drop until dissolution of the precipitate)
Add sodium carbonate up to a final volume of 750 ml.









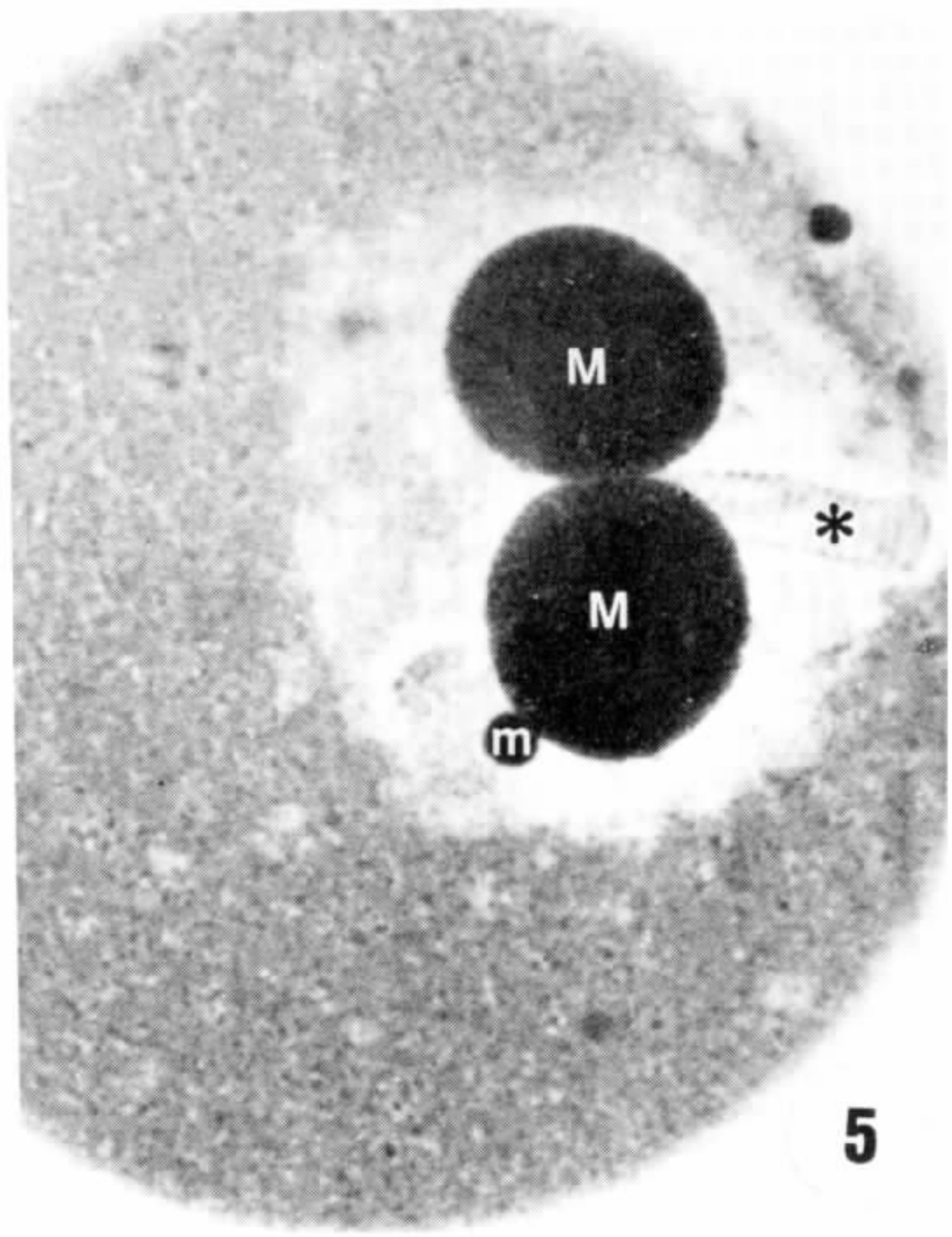


Fig. 1: ventral surface of the trophic form. Figs 2, 3: detail of the ventro-anterior region of the cell (Fig. 2) and lateral surface in the area of implantation of the marginal cirri (Fig. 3). Figs 4, 5: ciliary organelle reorganization during excystation.
*adoral zone membranelles; oral membranes (arrowhead); fronto-ventral-transverse cirri (white arrow); marginal cirri (black arrow); macronucleus (M); micronucleus (m) objectives: 40X (Figs 1, 4, 5); 100X (Figs 2, 3).

visualized in the cyst during excystation (Figs 4, 5). In an early excystment stage (Fig. 4), when there was just a single macronuclear mass, we identified collections of membranelles of the future adoral zone. Additionally, oral membranes and marginal rows of cirri were also seen. During a more advanced stage of the excystment process (Fig. 5), indicated by the fission of the single macronucleus into two independent macronuclear masses, the membranellar pattern of the adoral zone was seen to be already established.