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Morphological, Biometric, and Morphogenetic Comparison of Two Closely Related Species, *Stylonychia vorax* and *S. pustulata* (Ciliophora: Oxytrichidae)[1]

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ABSTRACT. Differences in the morphology of *Stylonychia vorax* Stokes, 1885 and *S. pustulata* (Müller, 1786) Ehrenberg, 1838 recognizable in vivo are the shape, the ventral cirral pattern, the caudal cirri, and the mode of moving. The dorsal-bristle complexes are distinguishable by the length of dorsal kinety four and the spaces among the pairs of basal bodies. When the ranges of variation of different populations and clones are compared by biometric analyses, *S. vorax* shows a relatively stable cortical pattern whereas in *S. pustulata* the cortical elements are regulated depending on the size of the body and the number of adoral membranelles. In *S. vorax* morphogenesis begins with a proliferation of basal bodies close to the transverse cirri. In contrast, in *S. pustulata,* the oral primordium appears de novo between the left marginal row and the postoral cirri. All other morphogenetic events are the same for both species. In proters and opisthes the six anlagen of the frontal-ventral-transverse cirri are of different origin and evolve independently. Three anlagen of the opisthe separate from the oral primordium, two originate from the right, and one from the left postoral cirrus. Three anlagen of the proter evolve from the posteriormost cirrus in the frontal area, one from the parental undulating membranes, one from the buccal cirrus, and one from the cirrus below the buccal cirrus. The anlagen one to six generate one, three, three, three, four, and four cirri. The characteristic arrangement of the undulating membranes and the participation of only two postoral cirri in the formation of primordia provide features that distinguish between the often confused genera *Oxytricha* and *Stylonychia.*

HYPOTRICHOUS ciliates of the genus *Stylonychia* are widespread and have been known for a long time (12). The discrimination of species is very difficult because former observers perceived few details in the living material with their instruments, and modern authors frequently illustrate nothing but the infraciliature. To better discriminate two known species, we redescribe the morphology of two closely related members of this genus, *S. vorax* Stokes, 1885 and *S. pustulata* (Müller, 1786) Ehrenberg, 1838 in vivo and with the aid of silver impregnation. Included is a careful biometric characterization and a comparison of morphogenetic events.

The nuclear behavior of this genus is widely investigated (1, 6, 21, 26, 31, 38, 43) and some species are used for experimental and biochemical research (7–9, 16, 25, 26, 29, 31, 35, 39, 40). Despite this extensive use as an experimental organism, as yet only single stages of the cortical development during binary fission have been described (16, 18, 19, 22, 23, 33, 37, 44–46). Such isolated stages can probably provide interesting insight into the systematics of higher taxa (e.g. families), but in our opinion they are almost useless for comparing species and genera because species and genera are often separated by minor—but none the less important—details.

MATERIALS AND METHODS

Two populations of *Stylonychia vorax* were investigated. They were taken from pasture pools of the Schlossalm (Bad Hofgastein, Salzburg) which were clearly separated by a ridge. *Stylonychia pustulata* was collected from Mondsee (Salzburg, Austria). Extremely small and large specimens were isolated for cloning. As a culture medium, tap water was used, with yeast and a species of the *Tetrahymena pyriformis* complex grown on dry egg yolk added as the food supply. Ciliates were examined carefully in vivo (bright field) soon after collection. The protargol staining method according to Foissner (14) was used to reveal the infraciliature. All measurements were performed using an ocular micrometer. With an oil immersion objective, one scale mark corresponded to 1.3 μm. All data in the tables are based on protargol-impregnated specimens sampled at random. Note that this staining method generally causes a shrinkage of about 20–30%.

All statistical procedures follow methods described in Sachs (36). Calculations were performed on a TI-59 minicomputer of Texas Instruments.

To make plain the changes during morphogenetic processes, old cirri were depicted only by contour whereas the new ones were filled in.

The terminology is according to Wallengren (46), Kahl (28), Borror (3), and Hemberger (23).

RESULTS

Morphology of the Non-Dividers

Stylonychia vorax Stokes, 1885. Body stiff, in vivo 93–137 × 48–55 μm. Anterior end rounded, posterior end tapering. Right margin convex, left one straight and obtusely pointed posteriorly (Fig. 1). About 3:1 flattened dorso-ventrally, first and last quarter very thin (Fig. 2). Macronuclear segments two, ovoid, in vivo 17 × 12 μm, both lying along the median or slightly right of it, filled with medium-sized masses ($\bar{x}$ = 2 μm in diameter, n = 12), probably nucleoli because they stain with protargol. Micronuclei two, spherical, in vivo 4 μm in diameter, each in close contact with one of the macronuclear segments. Contractile vacuole in vivo 13 μm, on the left-hand border above the middle of the body. Pellicle and endoplasm colorless. Sometimes yellow granules, about 2 μm in diameter, and dark inclusions, 3 × 1 μm, in the cytoplasm. Food vacuoles, in vivo 9 μm, content brown, green, or orange-yellow. Found in company with bacteria, green algae, *Colpidium, Chilodonella,* and *Prorodon.* Movement rapid, restless hasty turns, climbing upon the particles of mud by fits and starts, disturbed specimens exhibit rotation around the longer axis of the body. Conjugation was observed very frequently.

Marginal cirri in vivo 17–20 μm long, and going backward, the bases and the distances among them become smaller. On the right-hand side the cirri begin at the level of the buccal cirrus, all situated next to the body margin (Fig. 3). The left marginal cirri are displaced inwards anteriorly; marginal rows never confluent posteriorly. Three anterior frontal cirri enlarged, in vivo about 23 μm long. Two pairs of posterior frontal cirri separated by a gap. One buccal cirrus. Adoral zone of membranelles extends to the center of the ventral surface. After protargol staining the longest bases of the adoral zone of membranelles measure 15 μm. Paroral and endoral membranes straight, closely spaced, both composed of at least two rows of basal bodies. Very long pharyngeal fibers. Two morphogenetically

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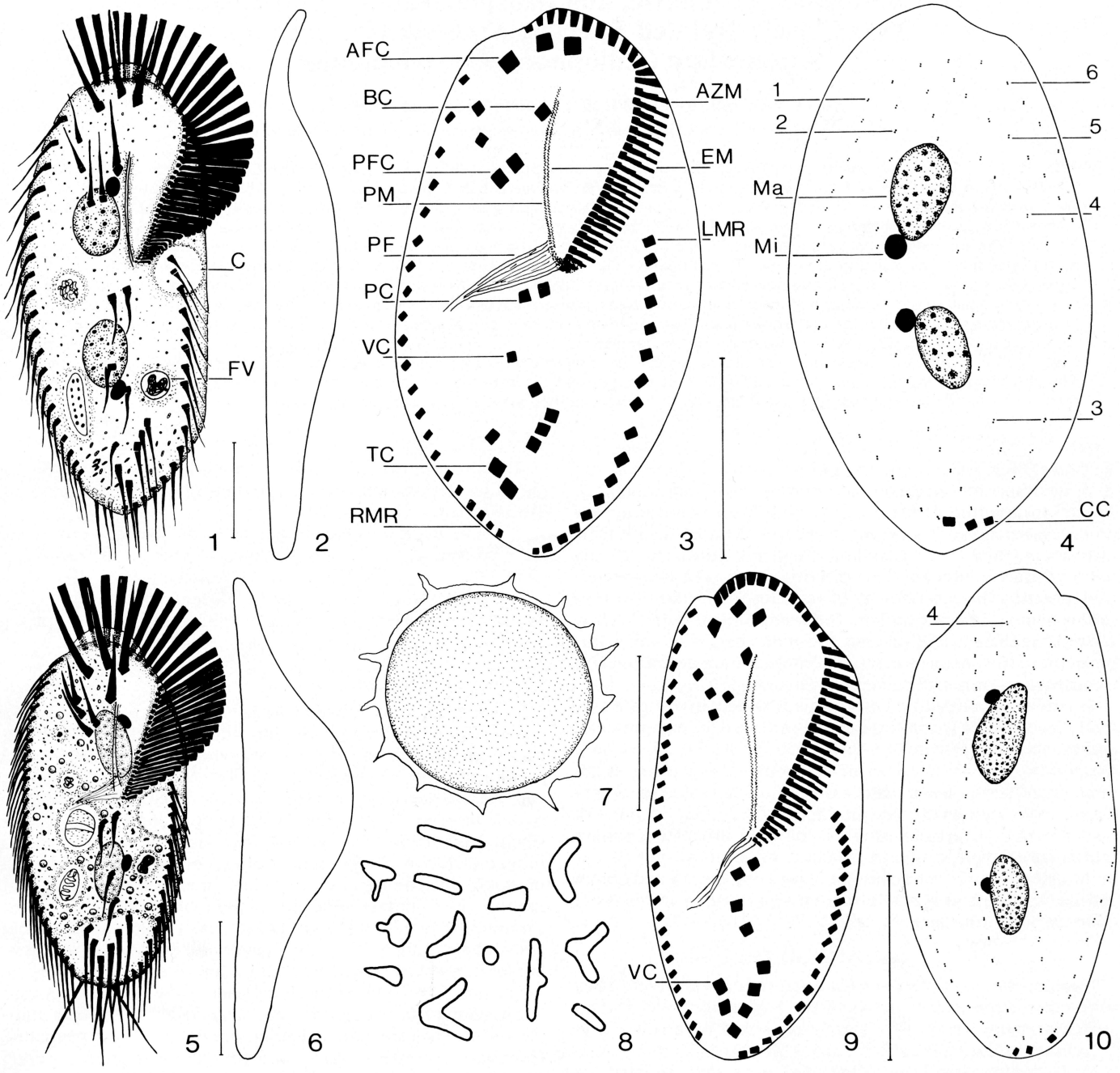


Figs. 1-10. Morphology of the non-dividing specimens. Figs. 1-4, *Stylonychia vorax*; Figs. 5-10, *S. pustulata*; Figs. 1, 2, 5-8, from life; Figs. 3, 4, 9, 10, infraciliature after protargol impregnation; Figs. 1, 3, 5, 9, ventral view; Figs. 2, 6, lateral view; Figs. 4, 10, dorsal view; Fig. 7, cyst; Fig. 8, inclusions in the cytoplasm. Each scale mark is equivalent to 30 μm. Legend: AFC, anterior frontal cirri; AZM, adoral zone of membranelles; BC, buccal cirrus; C, contractile vacuole; CC, caudal cirri; EM, endoral membrane; FV, food vacuole; LMR, left marginal row; Ma, macronucleus; Mi, micronucleus; PC, postoral cirri; PF, pharyngeal fibers; PFC, posterior frontal cirri; PM, paroral membrane; RMR, right marginal row; TC, transverse cirri; VC, ventral cirri; 1–6, number of dorsal kineties.

distinguishable postoral cirri at the end of the adoral zone of membranelles, behind them three smaller ventral cirri. Transverse cirri five, in vivo 17–21 μm long, characteristically arranged in a group of three cirri forming an oblique row, hardly reaching the posterior end of the body, and a detached group of two cirri protruding beyond the posterior border.

Dorsal kineties six (Fig. 4), cilia in vivo 3 μm long. The first three kineties as long as the body, composed of 18–24 ($\bar{x}$ = 21, n =5) pairs of basal bodies. One shorter row and two kineties which terminate before the anterior half of the body. Caudal cirri three, 19–22 μm long, unfringed, in vivo hardly distinguishable from the cirri of the marginal rows, adjacent to each other.

Stylonychia pustulata (Müller, 1786) Ehrenberg, 1838. Body

TABLE I. *Biometric characterization of* Stylonychia vorax *Stokes, 1885 and* S. pustulata *(Müller, 1786) Ehrenberg, 1838.*

	P1[a] (n = 28)					P2 (n = 25)					NP (n = 25)					SC (n = 25)					LC (n = 25)				
Character	$\bar{x}$	SD	CV	Min	Max	$\bar{x}$	SD	CV	Min	Max	$\bar{x}$	SD	CV	Min	Max	$\bar{x}$	SD	CV	Min	Max	$\bar{x}$	SD	CV	Min	Max
Body, length	80	6	8	70	91	91	8	8	75	110	65	13	21	43	90	79	8	10	64	93	94	9	10	79	108
Body, width	42	4	11	36	52	51	6	11	42	62	34	6	18	22	50	36	5	15	27	48	47	6	13	35	62
Ma, length	14	1	8	12	17	14	2	14	12	17	14	2	16	9	17	15	2	13	10	20	18	5	28	10	28
Ma, width	7	1	15	4	9	7	1	13	5	9	7	1	14	5	8	7	1	14	5	8	9	2	17	7	13
AZM, length	40	2	5	36	44	44	4	10	35	53	35	6	18	25	44	42	4	10	31	48	49	4	9	40	56
Number of AM	37	2	5	32	40	34	2	7	30	39	34	5	15	24	42	34	2	6	31	37	38	2	6	34	42
Number of LMC	17	1	5	15	18	17	1	6	15	19	19	2	13	12	22	19	2	9	16	22	21	2	8	18	24
Number of RMC	19	2	10	14	22	19	2	8	16	21	26	3	13	18	32	27	2	6	25	30	31	2	5	28	34

[a] All data are based on protargol-impregnated specimens. All measurements in μm. Legend: AM, adoral membranelles; AZM, distance from the anterior end of the body to the end of the adoral zone of membranelles; CV, coefficient of variation in %; LC, clone of a large specimen of *Stylonychia pustulata*; LMC, left marginal cirri; Ma, macronuclear segment; Max, maximum; Min, minimum; n, sample size; NP, natural population of *S. pustulata*; P1, population 1 of *S. vorax*; P2, population 2 of *S. vorax*; RMC, right marginal cirri; SC, clone of a small specimen of *S. pustulata*; SD, standard deviation; $\bar{x}$, mean.

inflexible, elliptical, in vivo 48–124 × 26–83 μm. Both ends rounded, margins more or less parallel (Fig. 5). About 2:1 flattened dorso-ventrally, central part often very bulged (Fig. 6). Macronuclear segments two, ovoid, in vivo 16 × 7 μm, lying left of the median, filled with numerous little masses ($\bar{x}$ = 0.8 μm in diameter, n = 12). Micronuclei two, in vivo 4 × 3 μm, each in close contact with one of the macronuclear segments. Contractile vacuole, in vivo 10 μm, on the left-hand border above the middle of the body. Endoplasm full of bright yellow, refringent inclusions, 2–3 μm, of various forms (Fig. 8). Food vacuoles, in vivo up to 32 μm, include diatoms sometimes reaching one-third of the body length. Even at low magnification the specimens, except for the peristome field and the area around the transverse cirri, show a dark color. Found in company with algae, *Stylonychia mytilus, Paramecium bursaria,* and *Coleps.* Movement moderately slow, gliding, and often standing still for some time, rotation around the longer axis on disturbance. Conjugation was observed very infrequently. Mixed clones in most cases encysted, but never showed pairing. Cysts have a toothed surface and a fine, bright endoplasm (Fig. 7).

Marginal cirri in vivo about 15 μm long, all bases are nearly of the same size, 1.5 × 0.7 μm, and equally spaced within the posteriorly clear-cut rows. Right marginal row begins at the level of the first adoral membranelles and is composed of many more cirri than the left one (Fig. 9). Three anterior frontal cirri enlarged, in vivo 19–23 μm long, bases about 2.6 × 2.6 μm. Four posterior frontal cirri, arranged in an oblique hook-shaped row, and one enlarged buccal cirrus. Adoral zone of membranelles often longer than half of the body length. After protargol staining the bases of the longest membranelles measure 22 μm. Undulating membranes straight and never overcrossing. Pharyngeal fibers distinguishable in vivo. Two postoral and three ventral cirri, from which the posteriormost one is adjacent to the right transverse cirrus. Transverse cirri five, in vivo 20–24 μm long, characteristically arranged in a group of four cirri in an oblique row adjoined by the fifth transverse cirrus and the equal-sized ventral cirrus. All transverse cirri project beyond the posterior border.

Six dorsal kineties (Fig. 10). Four long rows composed of 19–29 pairs of basal bodies ($\bar{x}$ = 26, n = 5). Note that kinety 4 extends to the anterior end of the body, contrary to *S. vorax* (Fig. 4). Two very short rows in the frontal area. Caudal cirri three, in vivo 16–25 μm long, unfringed, very stiff, projecting laterally. The distance between the two right caudal cirri is always smaller than that between the second and third ones.

Biometric Analyses

Two populations of *Stylonychia vorax* and one population and two clones of *S. pustulata* were studied quantitatively (Table I). Both species constantly show two macronuclear segments, two micronuclei, 18 cirri on the ventral face (except the marginal cirri), six dorsal kineties, and three caudal cirri. The coefficients of variation are strikingly low in the two populations of *S. vorax,* in contrast to those of *S. pustulata.* The relations—body length: body width and body length: length of adoral zone of membranelles—are very similar for all populations. The right marginal row is on average composed of two cirri more than the left one in *S. vorax* whereas there are eight cirri more in *S. pustulata* (Table I). The unique feature with no overlapping in the two species is the number of right marginal cirri; thus it is obviously the best criterion to separate these species in protargol slides. Both populations of *S. vorax* are very similar in their ranges of variation. The widest ranges of variation are to be found in the population of *S. pustulata.* Its two clones exhibit different behavior, the smaller one resembles the population of *S. pustulata,* the larger one is in most features more similar to the populations of *S. vorax* (Table I).

To discover significant differences we have examined the morphometric data by the non-parametric variance analysis of Kruskal & Wallis and the distribution-free multiple comparison of Nemeny (both in Sachs [36]) (Table II). The populations of *S. vorax* show significant differences in the body length and width, length of adoral zone of membranelles, and the number of adoral membranelles. The population of *S. pustulata* differs from both populations of *S. vorax* in nearly all characters. The small clone is extremely similar to the population of *S. pustulata,* from which it was isolated. The large clone is a kind of mixture of the two species, except in the number of right marginal cirri (Table II).

Stylonychia vorax and *S. pustulata* show different behavior in the regulation of the cortical pattern (Table III). In the population and the small clone of *S. pustulata,* the pairs of variables are correlated in a constant and characteristic way. They contrast in this respect with *S. vorax,* where the biometric parameters are nearly independent from the body length and the number of adoral membranelles.

Cortical Development During Cell Division

Stomatogenesis. In *Stylonychia vorax* small groups of basal bodies evolve very close to the intact uppermost one or two

TABLE II. *Distribution-free multiple comparison of different populations and clones of* Stylonychia vorax *and* S. pustulata.

BL[a]	P2	NP	SC	LC
P1	1009**[b]	596	37	937**
P2		1605**	1046**	128
NP			559	1732**
SC				1173**

AM	P2	NP	SC	LC
P1	914**	822*	1040**	220
P2		92	126	1133**
NP			218	1041**
SC				1259**

BW	P2	NP	SC	LC
P1	863**	1055**	769*	453
P2		1917**	1632**	410
NP			286	1508**
SC				1222**

LMC	P2	NP	SC	LC
P1	190	1348**	1064**	1888**
P2		1158**	874**	1698**
NP			284	540
SC				824*

AZM	P2	NP	SC	LC
P1	844**	379	564	1508**
P2		1223**	280	664
NP			943*	1887**
SC				944**

RMC	P2	NP	SC	LC
P1	87	1509**	1355**	2080**
P2		1596**	1441**	2166**
NP			155	571
SC				725*

[a] See legend to Table I; BL, body length; BW, body width.
[b] Differences greater than 699 are significant at the 0.05 level (*); those over 834 are significant at the 0.01 level (**).

transverse cirri of the left group (Fig. 11, arrow). In *S. pustulata* the oral primordium originates de novo between the left marginal row and the postoral cirri (Fig. 12, arrow). The proliferation of new basal bodies consequently proceeds forward in *S. vorax* and toward the posterior area in *S. pustulata.* In both cases a large anarchic field is produced (Fig. 13). The mode of formation of the oral primordium is the unique difference in cortical morphogenesis that we found between the populations of the two species. Therefore the following drawings are valid for *S. vorax* as well as for *S. pustulata.* Only the first third of the parental undulating membranes is reorganized (Figs. 16–19) because no further dispersal of basal bodies occurs. The primordium for the undulating membranes of the opisthe is formed by a group of basal bodies extending toward the anterior right of the oral anarchic field (Fig. 14). At this time also, the first new adoral membranelles appear. The pharyngeal fibers of the proter are not visible with protargol in later stages of the morphogenesis (Figs. 20, 21); probably they are reorganized.

Development of the cirral primordia. The frontal-ventral-transverse (FVT)-anlagen develop in chronological order as follows: three FVT-anlagen in the proter originate from the lowest of the posterior frontal cirri (Figs. 14 arrow, 15). At the same time three FVT-anlagen in the opisthe separate from the oral primordium (Fig. 14). Two anlagen evolve from the dispersion of the right and one from the left postoral cirrus (Figs. 15, 16). In the proter, additional basal bodies from the undulating membranes, the buccal cirrus, and the left posterior frontal cirrus participate in the formation of primordia (Fig. 16). Uniformly, six FVT-anlagen are produced in each filial product (Figs. 17, 18). The development of the marginal primordia always starts within the right row and proceeds more rapidly than those of the left row in the earlier stages of binary fission (Figs. 16, 17). Later, the events in the marginal rows appear to be correlated (Figs. 18–21). The remaining 13 ventral cirri and several marginal cirri will be resorbed in the course of the division.

Differentiation of new cirri. The constant set of 18 cirri is formed within the six FVT-primordia as follows (Fig. 18):

Anlage	1	2	3	4	5	6
Number of cirri	1	3	3	3	4	4

The considerable displacement of these 18 cirri during morphogenesis (Figs. 18–21) is obviously not completely identical in the two species because of the already mentioned differences in the arrangement of the posterior frontal cirri and the transverse cirri in the non-dividing specimens (Figs. 3, 9). "True" transverse cirri are formed at the posterior tip of the anlagen 2–6.

Development of the dorsal primordia. Three anlagen originate within the parental kineties 1, 2, and 3 (Fig. 22). They generate four dorsal kineties (Figs. 23, 24). The two short kineties develop next to the right marginal row (Figs. 19, 20) and are displaced to the dorsal face later (Figs. 23, 24). Caudal cirri differentiate at the posterior tip of kineties 1, 2, and 4 (Figs. 23, 24). Electron micrographs of these events appear in Grimes & Adler (18).

The division of the nuclear apparatus (Figs. 23, 24) is according to Summers (43) and Sapra & Dass (37).

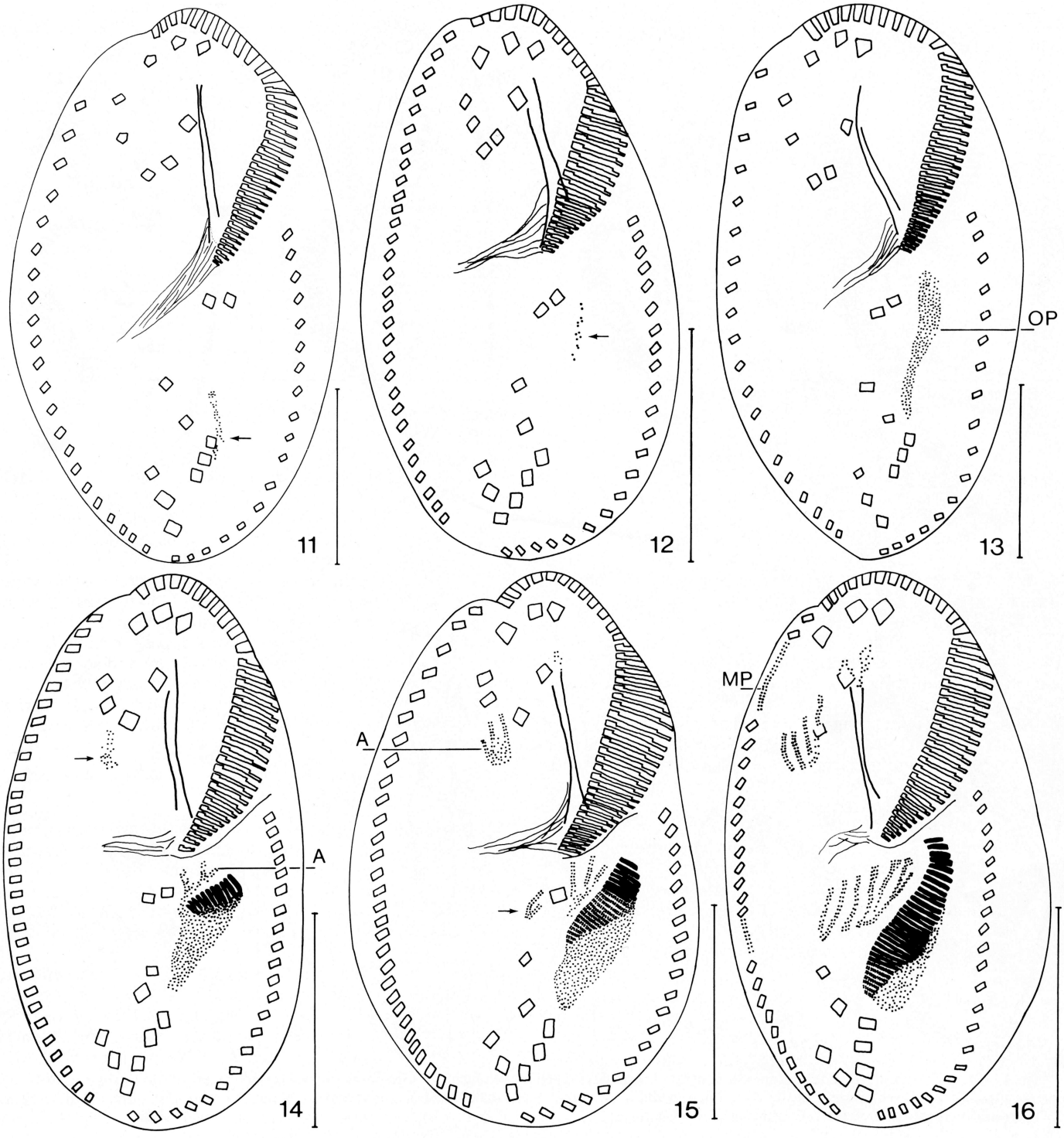
TABLE III. *Coefficients of rank correlation (Spearman) and probability of error (P[a]) for biometric parameters in* Stylonychia vorax *and* S. pustulata.

Pairs of variables	P1[b]	P2	NP	SC	LC
Length/width	0.75***	0.38*	0.79***	0.75***	0.63***
Length/AZM	0.27 ns	0.71***	0.94***	0.78***	0.47***
Length/AM	0.10 ns	0.53**	0.83***	0.70***	0.26 ns
Length/LMC	0.37 ns	0.30 ns	0.83***	0.76***	0.42*
Length/RMC	0.20 ns	0.28 ns	0.87***	0.62***	0.51**
AM/LMC	0.11 ns	0.24 ns	0.70***	0.59***	0.29 ns
AM/RMC	0.11 ns	0.08 ns	0.78***	0.58***	0.58**

[a] Significance of differences against zero. Legend: ns, nonsignificant; *, $0.05 \leq P \geq 0.01$; **, $0.01 \leq P \geq 0.001$; ***, $P \leq 0.001$.
[b] See legend to Table I.

DISCUSSION

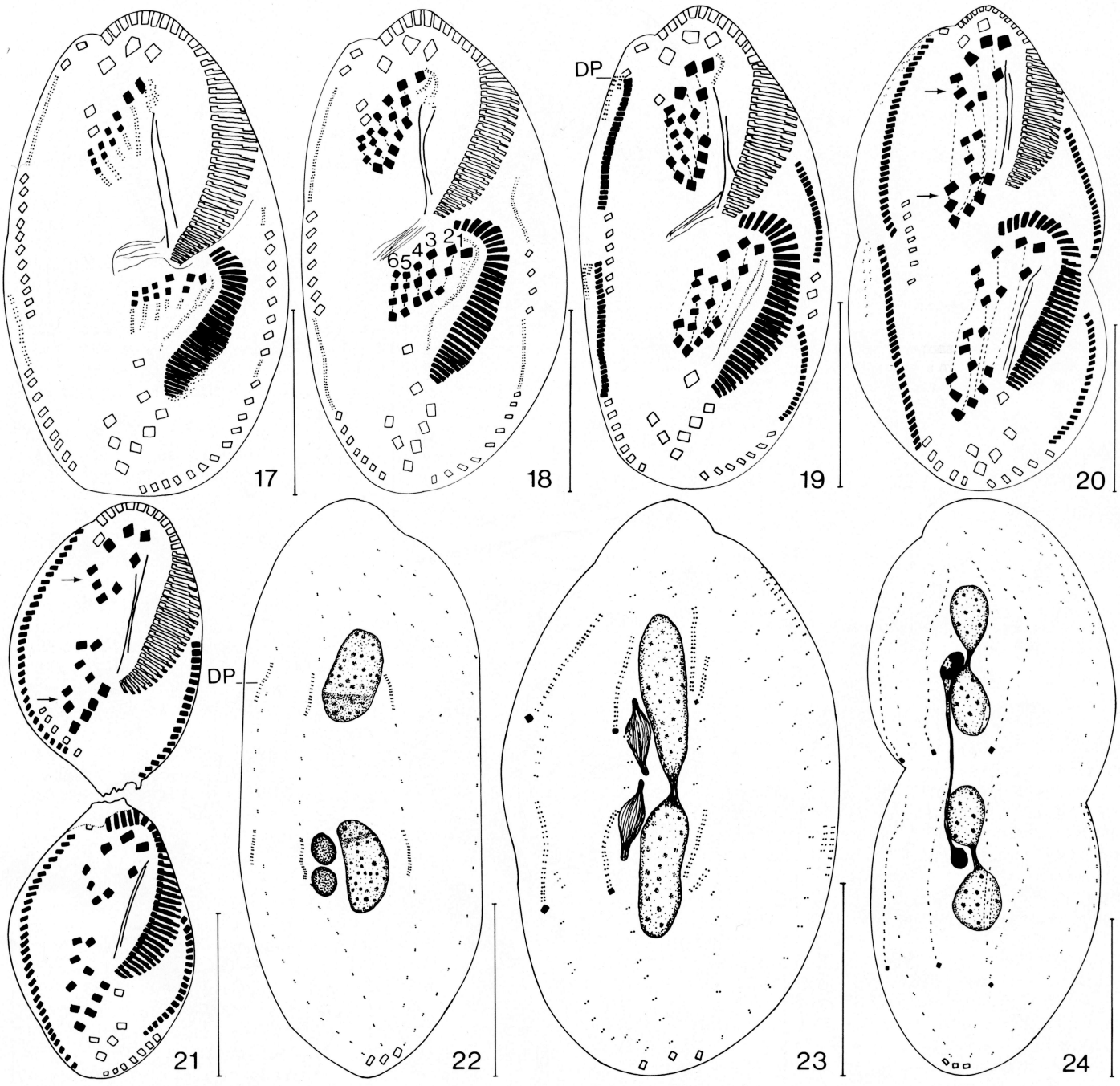
Identification of species. Numerous species descriptions of the genus *Stylonychia* have been published but in modern revisions only seven (3) or 14 (23) species names are considered to be valid. Based on 27 original descriptions, the identification of our species was somewhat bewildering. In ciliate taxonomy, the fundamental importance of in vivo observations—often treated too superficially in modern times—along with the description of the infraciliature and, if possible, cortical morphogenesis,



Figs. **11-16**. Morphogenetic stages in ventral view. Fig. **11**, first stage in *Stylonychia vorax*: Fig. **12**, first stage in *S. pustulata*. Arrows point to the OP; Fig. **13**, early stage in *S. vorax*; Figs. **14-16**, middle stages in *S. pustulata*. Arrow in Fig. **14** points to the disintegrated posteriormost frontal cirrus, which in Fig. **15** marks the disintegrated right postoral cirrus. Each scale mark is equivalent to 30 μm. Legend: A, frontal-ventral-transverse-anlage; MP, marginal primordium; OP, oral primordium.

cannot be over-emphasized, as Foissner & Adam (15) have recently stressed.

Stylonychia vorax was identified in accordance with Stokes (42). In our opinion, synonyms are *Histrio hyalinus* Vörosvary, 1950, and *Stylonychia putrina* (47), based on the body shape, the arrangement of cirri, and the position of the undulating membranes. The same is true for the smaller variety of *S. putrina* (11) because of the group of three and two transverse cirri,



Figs. 17-21. Late morphogenetic stages in ventral view of *Stylonychia pustulata*. Arrows point to the displaced cirri of the farthest right FVT-anlage. Figs. 22-24. Development of the dorsal primordia in *S. vorax*. Each scale mark is equivalent to 30 μm. Legend: DP, dorsal primordium; FVT, frontal-ventral-transverse; 1–6, number of FVT-anlagen.

the number of adoral membranelles, and left and right marginal cirri. Unlike Borror (3) and Hemberger (23), we consider *S. ovalis* Dragesco, 1966 as valid on the basis that it shows a greater number of adoral membranelles and marginal cirri than *S. vorax*.

Stylonychia pustulata was identified by the shape of the body (12, 28, 33, 43), the arrangement and the number of cortical elements, the mode of moving (34), the dorsal-bristle complex (18), the toothed cysts (13), and the typical inclusions (10, 20, 25, 34). We agree with Borror (3) that *S. fissiseta* Claparède & Lachmann, 1858, which Hemberger (23) transferred to *S. mytilus*, is a synonym of *S. pustulata*. Both consider *S. notophora* Stokes, 1885 to be indistinguishable from *S. pustulata*. We agree because of its morphological similarities and the de novo stomatogenesis, described in (37); however, some authors (29, 31, 39) reported four micronuclei in this species.

The characters for separating these two closely related species are summarized in Table IV.

TABLE IV. *Criteria for the discrimination of* Stylonychia vorax *and* S. pustulata.

Character	*Stylonychia vorax*	*Stylonychia pustulata*
Origin of the oral primordium	close to one or two TC[a]	de novo
First right marginal cirrus	at the level of the buccal cirrus	below the first adoral membranelle
Relation of RMC and LMC	two RMC more than LMC	eight RMC more than LMC
Posterior frontal cirri	two pairs separated by a gap	one oblique, hook-shaped row
Transverse cirri	two detached groups of three and two TC	four TC in an oblique row, adjacent one TC and one ventral cirrus
Dorsal kineties	shorter kinety 4	four long kineties
Pairs of dorsal bristles	in a loose arrangement	closely spaced
Caudal cirri	closely spaced, hardly distinguishable from marginal cirri	left one set apart, conspicuously projecting laterally
Longest bases of AM	15 μm	22 μm
Shape of the body (in vivo)	posterior end tapering	parallel borders, both ends rounded
Movement	rapid and restless with hasty turns	gliding and often standing still for some time

[a] Legend: AM, adoral membranelles; LMC, left marginal cirri; RMC, right marginal cirri; TC, transverse cirri.

Biometric comparison. Stylonychia vorax shows relatively stable morphological characters, well documented by the low coefficients of variation (Table I). This stability is strengthened by the independence of cortical structures from the body length and the number of adoral membranelles (Table III). The polymorphism reported for *S. pustulata* and *Paraurostyla weissei* (27, 48) is striking in comparison. The morphological parameters of *Stylonychia pustulata* show wide ranges of variation, also shown by the distinct variability of shape (41). Zingher et al. (49) found in natural populations of this species variations from 74 × 34 to 166 × 81 μm, in clones variations from 61 × 29 to 116 × 57 μm. These data compare well with ours. In aged cultures, cells were observed (29) with a body length of 10 μm and macronuclear abnormalities as we have observed. Lynn & Malcolm (32) investigated the morphometric variation of 19 clones from eight species of the ciliate genus *Colpoda.* They supposed for *C. cucullus,* which has a clonal mean of somatic length between 40 and 70 μm, that analysis of isoenzyme variation would clearly reveal the occurrence of sibling species. In the meantime species status was given to the syngens of *Stylonychia mytilus,* formerly called varieties, by enzyme electrophoresis (2, 40). In *S. pustulata* there are three varieties (35); therefore we expect that it is also a complex of sibling species. We tried to determine the mating-type reactivity by mixing about one dozen different clones in pairs, but most of the specimens encysted without showing conjugation. Fermor (13) got the same results; within three months his *S. pustulata* encysted twice but never conjugated. Ivanić (26) supposed that encystment in *S. pustulata* has special internal conditions. This is supported by our observations on *S. vorax,* which conjugated frequently but never encysted. No one else has ever described encystment in *S. vorax.* In *S. notophora,* which seems to be a synonym of *S. pustulata,* conjugation can be induced easily (38). In our opinion the mating reactions must be used extremely carefully in determining sexual isolation and assigning species status, because there are too many unknown factors and, moreover, environmental and physiological conditions can influence sexual behavior. For instance, Ammermann & Schlegel (2) reported that some strains of *S. mytilus* and *S. lemnae* conjugate with each other in the laboratory.

The clone of large *S. pustulata* differs in some characters and in the regulation of cortical pattern from the population and the clone of small *S. pustulata* (Tables II, III). Because of the numerous overlappings and the identical morphogenesis we are convinced that the existing differences do not provide enough reason for a consideration of these strains as separate species. Similar results were obtained on several species of *Trichodina* (30): within each of the examined species great and statistically significant differences exist among particular populations. In the genus *Euplotes* extensive variation, even among the progeny of a single individual, was discovered (17).

Morphogenetic processes. As yet no unequivocal proof has been furnished for apokinetal stomatogenesis in *Stylonychia* (19, 23, 37, 44). Our results show the earliest stages (Figs. 11, 12) and verify the different places of the origin of the oral primordium in this genus. This provides further evidence for Foissner & Adam's conclusion (15) regarding the genus *Oxytricha,* that the site of stomatogenesis cannot be used in the classification of Hypotrichida at the generic level.

Another striking feature of the investigated species and *Stylonychia mytilus* (23) is the involvement of only two postoral cirri in the development of anlagen whereas in other oxytrichous genera there are three (5, 15, 19, 23). This criterion gives us a definite means—besides the characteristic straight and parallel undulating membranes—of separating the frequently confused genera, *Oxytricha* and *Stylonychia.*

Concerning the development of the remaining cirral primordia, little detailed information is available on *Stylonychia.* For *S. histrio* very inexact comments are given about this event (22). In *S. mytilus* the buccal cirrus, the posterior left cirrus in the frontal area, and the third postoral cirrus do not participate in the formation of anlagen (23). *Stylonychia grandis* (23) is probably a member of the genus *Oxytricha* because of its crossed and curved undulating membranes. In *S. notophora* (37) the primordia are said to arise without any relationship with the basal bodies of the parental structures. We observed just the opposite behavior in our two species (Figs. 14–16).

By comparison with some oxytrichous genera (3–5, 11, 15, 19, 23, 37) we find that the two uppermost posterior frontal cirri (Figs. 20, 21, arrows) resemble the frontoterminal cirri (23) (named migratory cirri [4]) of the genus *Holosticha.* Possibly they are homologous, because in all cases they do not participate in the formation of primordia and they originate in the rightmost FVT-anlage. During morphogenesis these cirri shift to a position beside the anterior end of the right marginal row. The posterior cirri of this anlage are finally placed near the posterior end of the cell (Figs. 20, 21, arrows). Thus the holostichous and oxytrichous genera could be sister groups according to Hennig (24).

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