

## Physiological reorganization in the hypotrich ciliate *Apoamphisiella vernalis* (Protista, Ciliophora, Hypotricha)

Larissa A. M. de Castro<sup>1</sup>, Inácio D. da Silva-Neto<sup>2</sup>, Thiago da S. Paiva<sup>1,3</sup>

1. Laboratório de Biologia Molecular "Francisco Mauro Salzano", Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil. (tpaiva@biologia.ufrj.br)

2. Laboratório de Protistologia, Departamento de Zoologia, Instituto de Biologia, CCS, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

3. Laboratório de Protistologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil.

Received 15 January 2016

Accepted 7 December 2016

DOI: 10.1590/1678-4766e2017012

**ABSTRACT.** Physiological reorganization is described for the first time for the hypotrich ciliate genus *Apoamphisiella* Foissner, 1997, based on a population of *A. vernalis* (Stokes, 1887) Berger, 2006 collected from an eutrophic pond in the state of Minas Gerais, Brazil. Stomatogenesis is epiapokinetal, and the cirral pattern reorganizes from six typical fronto-ventral-transverse primordia, plus at least one short primordium located between IV and V. Primordia I and II originate from disaggregating undulating membranes and buccal cirrus, respectively; primordium III originates from left frontoventral cirrus plus a streak of basal bodies extending from the anterior end of oral primordium; primordium IV and the short extra primordia are formed from basal bodies perhaps associated to the posterior end of III plus the disaggregation of anteriormost cirri of the left ventral row. Primordia V and VI arise within the right ventral row as a single streak, splitting to form the two primordia. Marginal primordia develop initially within the pre-existent marginal rows, continuing to reorganize outside, pushing the old marginal cirri leftwards. Two dorsomarginal kineties develop associated to the right marginal row primordium. Dorsal ciliature also reorganizes from within the pre-existent dorsal kineties. The two macronuclear nodules approach each in middle reorganizers, briefly touching each other. At least one micronucleus undergo division. The process of physiological reorganization in *A. vernalis* resembles that of the North American *Paraurostyla weissei* complex representative, and along with features of the interphase morphology, indicate that *Apoamphisiella* belongs or is related to the *Cyrtohymena-Paraurostyla* group, within the Dorsomarginalia.

**KEYWORDS.** Atlantic Forest, Dorsomarginalia, morphogenesis, ontogeny, Spirotrichea.

The Hypotricha (= Stichotrichia *sensu* Small & Lynn, 1985) exhibit an astounding variety of ontogenetic processes that originate their highly diverse ciliature patterns (FOISSNER, 1996; FOISSNER *et al.*, 2002; LYNN, 2008). Investigation of ontogeny in hypotrichs is thus of major importance for elucidating their life cycles and to explain how the ciliature patterns develop and organize, facilitating detection of homologies and convergences (e.g. JERKA-DZIADOSZ, 1965, 1972; JERKA-DZIADOSZ & FRANKEL, 1969; BORROR, 1979; FLEURY, 1983; EIGNER, 1995, 1997, 1999, 2001; EIGNER & FOISSNER, 1992, 1993, 1994; BERGER & FOISSNER, 1997; PAIVA & SILVA-NETO, 2007, 2009; SIQUEIRA-CASTRO *et al.*, 2009; DE CASTRO *et al.*, 2016). ZOU & NG (1991) consider physiological reorganization as an ontogenetic process secondarily derived from divisional morphogenesis. It is responsible for renewing a complete conjunct of ciliary elements in intact, non-dividing individuals (GRIMES & ADLER, 1978). According to BERGER (1999), physiological reorganization usually occurs in response to unfavorable environmental (or culture) conditions, such as starvation or other more subtle changes in the medium. Likewise, very similar reorganizational processes happen to reestablish the normal ciliature after conjugation and excystment events take

place, and to regenerate the ciliature after physical trauma (e.g. DEMBOWSKA, 1925; 1938; 1939; NG, 1990; KAMRA & SAPRA, 1991; ZOU & NG, 1991; FRONTCZACK-BANIEWICZ & JERKA-DZIADOSZ, 1992).

In the present study, we describe, for the first time, stages of physiological reorganization in the hypotrich genus *Apoamphisiella* Foissner, 1997, based on a strain of *Apoamphisiella vernalis* (Stokes, 1887) Berger, 2006, from an eutrophic pond in the Atlantic Forest in the state of Minas Gerais, Brazil. The origins of the various elements of the ciliature are traced from ontogeny during physiological reorganization, and results are discussed in comparison with the literature on supposedly related species.

### MATERIAL AND METHODS

Specimens of *Apoamphisiella vernalis* were obtained from limnetic cultures enriched with crushed rice grains, made using water with sediments collected from a shallow eutrophic pond in the state of Minas Gerais, Brazil, in November of 2002 (approximate geographic coordinates: 21°59'36.4"S, 44°57'17.7"W). Specimens were identified using standard light and electron microscopy techniques commonly used for ciliate taxonomy, which included bright-

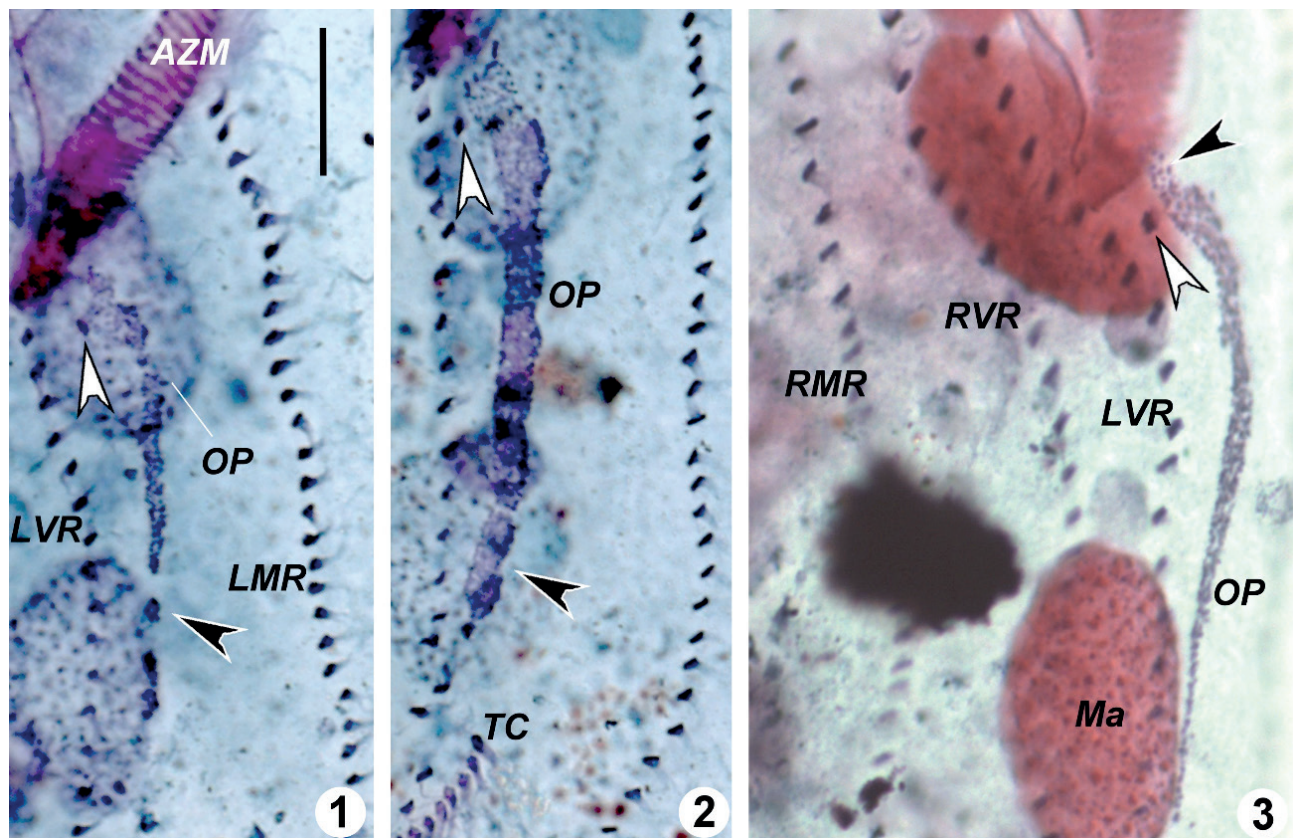
field, differential interference contrast microscopy, scanning electron microscopy and protargol-impregnation (DRAGESCO & DRAGESCO-KERNÉIS, 1985; DIECKMANN, 1995; DA SILVA-NETO *et al.*, 2012). The interphase (non-dividing) cells of this strain were studied in detail by PAIVA *et al.* (2014), thus the reader is referred to that work and the original description by STOKES (1887) for the general morphology of *A. vernalis*.

To study physiological reorganization, specimens from near starving cultures of approximately two to three weeks old were fixed Bouin's fluid and impregnated with protargol, following DIECKMANN (1995). Specimens were then mounted in permanent slides and analyzed under bright field microscopy at 500–1,000x. Scale bars are included in the photographs when necessary (FOISSNER & XU, 2006). Terminology follows WICKLOW (1982) (e.g. "procirri" to refer to immature cirri), BERGER (1999, 2006, 2008, 2011) and PAIVA *et al.* (2014); primordia numbering is based on WALLENGREN (1900) and DE CASTRO *et al.* (2016). Voucher slides are deposited in the collection of Laboratório de Protistologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro.

## RESULTS

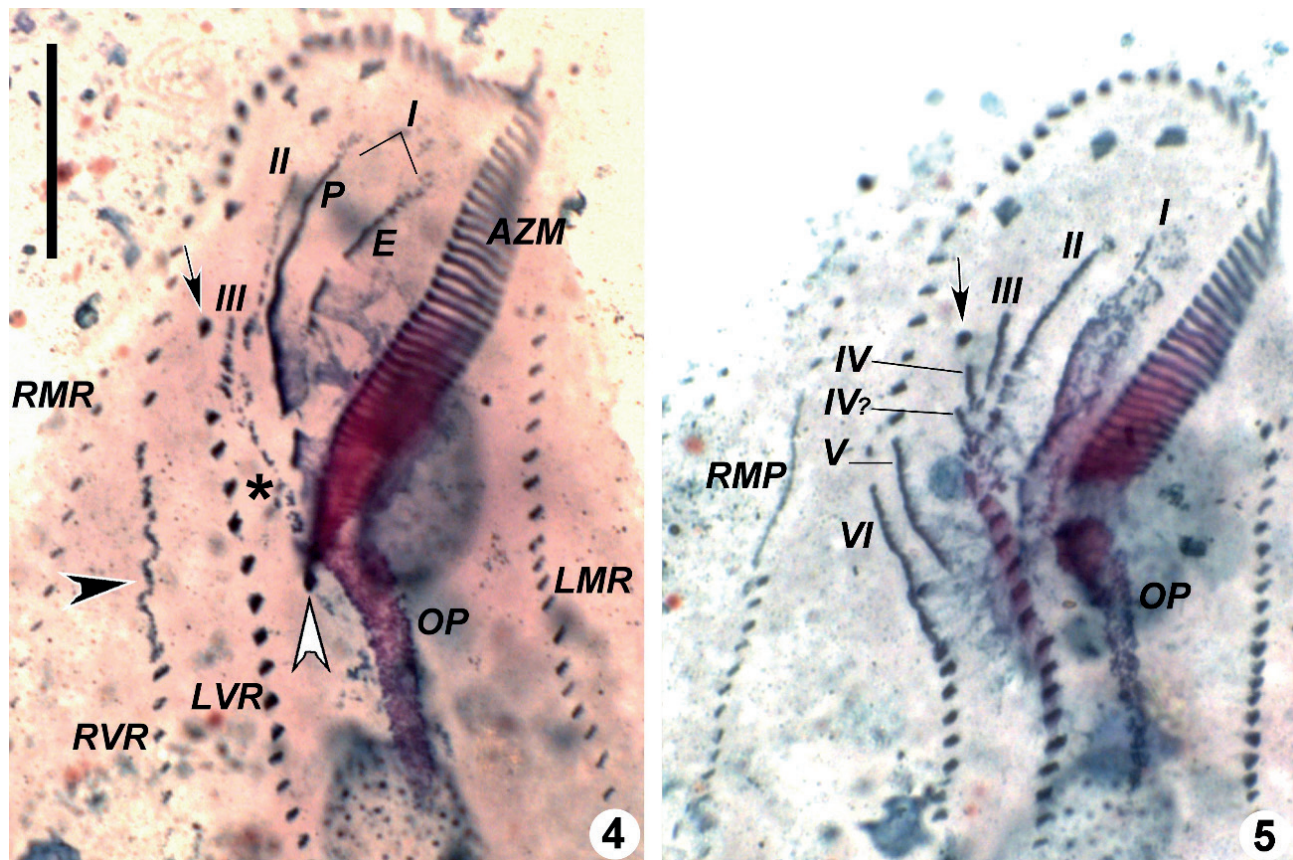
Physiological reorganization in *Apoamphisiella vernalis* begins with the formation of an elongated, likely epiapoknetal, oral primordium, which originates *de novo* in the space between

the left marginal and left ventral cirral rows. Such primordium is slender and slightly curved rightwards anteriorly. At its posterior region, there are small patches of basal bodies, suggesting the oral primordium arises from multiple points (Fig. 1). The oral primordium then becomes progressively elongated, forming a short anterior projection and growing slender posteriad (Figs 2, 3). Next, it becomes shorter and clavate, and then commences to produce new adoral membranelles, which will replace approximately half of the old lapel (Figs 4, 5). At the anterior region of body, the paroral and endoral membranes simultaneously dissociate. Starting distally, the disaggregation of basal bodies moves posteriad until both membranes form primordium I. The fronto-ventral-transverse (FVT) primordium II originates from the disaggregation of the buccal cirrus, and primordium III from a long streak that proliferates anteriorly from the anterior projection of oral primordium, fusing to the dissociating left frontoventral cirrus. Such long streak possibly contributes also to primordium II. At this stage, a streak of basal bodies which precedes primordia V and VI, grows within the right ventral row (Fig. 4). Primordia IV and IV<sub>2</sub> arise likely associated with basal bodies near the posterior region of III, with participation of one or two anteriormost cirri from the left marginal row. As primordium II grows posteriad, the five streaks form a fan-shaped structure. The right marginal primordium is already visible at this stage, growing initially within the original structure. Meanwhile, the streak of basal bodies within the right



Figs 1-3. Ventral side of early reorganizers, after protargol-impregnation. Figs 1, 2. Epiapoknetal stomatogenesis. Notice some discontinuities in the oral primordium (black arrowheads). Fig. 3. Oral primordium becomes curved to the right and forms an anterior projection (black arrowhead). The white arrowheads mark the postperistomal cirrus, which was not yet resorbed. (AZM, adoral zone of membranelles; LMR, left marginal cirral row; LVR, left ventral cirral row; Ma, macronuclear nodule; OP, oral primordium; RMR, right marginal cirral row; RVR, right ventral cirral row). Scale bar = 20  $\mu$ m.





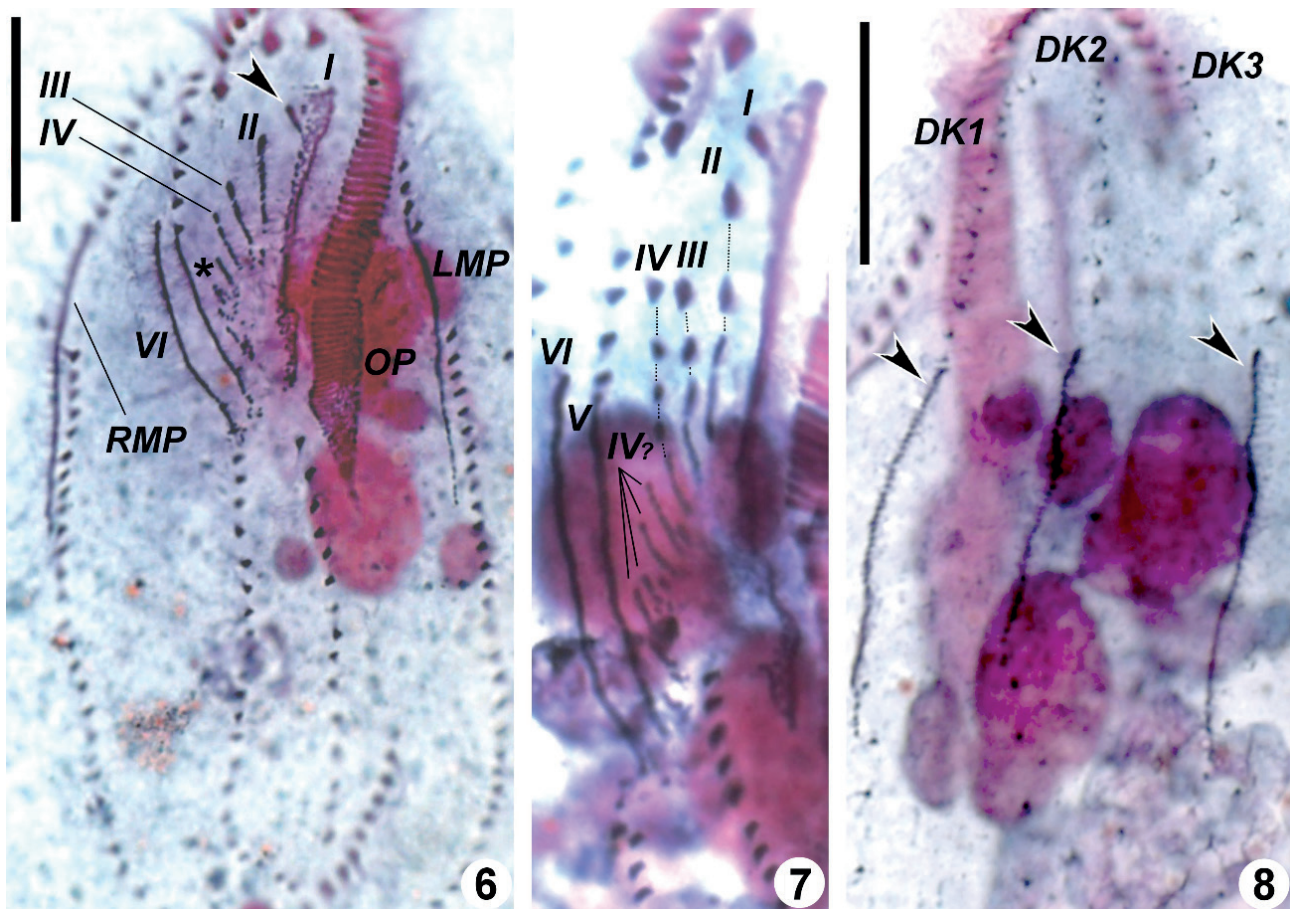
Figs 4, 5. Ventral side of early-to-middle reorganizers, after protargol impregnation. Fig. 4. Early development of primordia I–III. Black arrowhead shows the streak of basal bodies which will form primordia IV and V; white arrowhead shows the postperistomal cirrus. Notice a streak of basal bodies originating from anterior end of oral primordium (asterisk), which contributes to the formation of primordium III. Fig. 5. Early development of primordia IV–VI and right marginal primordium. The arrows point to the right frontoventral cirrus. Fronto-ventral-transverse primordia numbered in romans. (IV<sub>2</sub>, short primordia possibly related to IV; AZM, adoral zone of membranelles; E, endoral; LMR, left marginal cirral row; LVR, left ventral cirral row; OP, oral primordium; P, paroral; RMP, right marginal primordium; RMR, right marginal cirral row; RVR, right ventral cirral row). Scale bar = 20µm.

ventral row splits into primordia V and VI (Fig. 5).

In middle-reorganizers, the postperistomal cirrus have been resorbed, and possibly incorporated in the differentiating oral primordium. The right frontoventral cirrus may be resorbed in middle reorganizers or retained until late stages. The left marginal primordium originates at this stage, similarly to the primordium for the right marginal row. Both right and left marginal primordia elongate posteriad, outside and to right of the old structures, pushing them leftwards. Simultaneously, there appear some short basal body streaks continuous with the posterior region of the fan-shaped cluster of FVT primordia, located between IV and V, and forming a ladderized pattern. At this stage, the left frontal procirrus begins to differentiate rightwards from the distal end of the undulating membranes primordium, and the basal bodies of the oral primordium organize into new adoral membranelles (Figs 6, 7). As reorganization proceeds, the FVT cirral streaks begin to differentiate, with cirri maturing posteriad (Fig. 7). The dorsal primordia arise within the old structures (Fig. 8), and fragmentation of dorsal kinety 3 [which happens in divisional morphogenesis (DE CASTRO *et al.*, 2016) and is noticeable in interphase (PAIVA *et al.*, 2014)] possibly occurs in very late reorganizers.

In middle-to-late reorganizers, the short streaks of basal bodies that originate between FVT primordia IV and V, viz. primordia IV<sub>2</sub>, form each a single transverse cirrus (Figs 9, 10). During the process, they produce vestigial procirri, which are resorbed (Fig. 9). However, in some specimens (Fig. 10), one of such procirri in the leftmost IV<sub>2</sub> primordium can mature, becoming an extra postperistomal cirrus. Dorsomarginal kineties are formed right of right marginal row primordium, near its anterior end. The outer dorsomarginal kinety starts its development contiguously with the juvenile marginal cirri, then separates and moves rightwards. The paroral and endoral membranes appear differentiated, but still disposed parallel to each other, hence, the cyrtohymenid curvature and subsequent deepening of the oral cavity must occur in late reorganizers, which were not observed among the studied specimens (Fig. 10). During physiological reorganization, the distance between the macronuclear nodules is conspicuously reduced, the nodules briefly touching each other (Fig. 11). Fusion of the macronuclear nodules was not observed. At least one or two micronucleus per cell becomes ellipsoid and divides (Figs 11-14).

After reorganization, the remaining old ciliary structures are fully resorbed, except by the collar and



Figs 6-8. Middle reorganizers, after protargol-impregnation. Figs 6, 7. Ventral side of specimens showing primordia segregation in 6 and differentiation in 7. The arrowhead in Fig. 6 shows the left frontal cirrus differentiating from the undulating membranes primordium (I). An asterisk marks the ladderized arrangement of the short primordia IV<sub>s</sub>. Fig. 8. Dorsal side showing dorsal kineties primordia (arrowheads). Fronto-ventral-transverse primordia numbered in romans (IV<sub>s</sub>, short primordia possibly related to IV; DK(n), dorsal kineties; LMP, left marginal primordium; OP, oral primordium; RMP, right marginal primordium). Scale bars = 20µm.

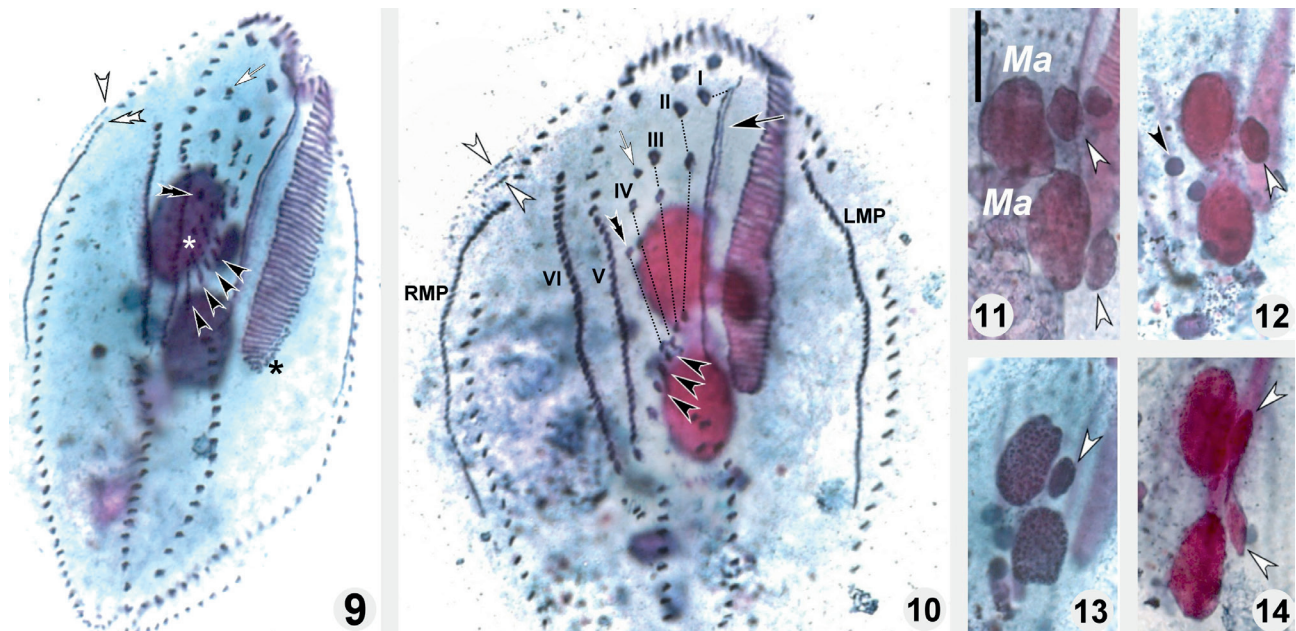
approximately half of the lapel adoral membranelles, which are maintained after physiological reorganization. The origins of each FVT primordia and their cirral products are summarized in Tab. I.

## DISCUSSION

Until now, the process of physiological reorganization in *Apoamphisiella* was never described. When compared to species of supposedly related genera, the reorganization in *Apoamphisiella vernalis* resembles that described by JERKA-DZIADOSZ & FRANKEL (1969) for a North American population of the *Paraurostyla weissei* complex, viz. *Paraurostyla polymicronucleata* Merriman, 1937, according to BERGER (1999). In both cases, the oral primordium is curved rightwards and originates *de novo* [vs. originates adjacently (parakinetal?) to postperistomal cirri row in the European *P. weissei* (WIRNSBERGER *et al.*, 1985)]; the undulating membranes start disaggregation before fusing; about half of the membranelles of the lapel are replaced; and the two rightmost FVT primordia originate from a primordium growing within the rightmost ventral row, which then splits in two.

Another important shared feature is the participation of basal bodies originating from the oral primordium to the formation of the FVT primordia. In *A. vernalis*, one long streak of basal bodies extends from the anterior end of the oral primordium and merges with the disaggregating left frontoventral cirrus, resulting in an small anarchic field of basal bodies near the posterior end of III, from where primordia IV and then IV<sub>s</sub> arise. Such long streak possibly also contributes to primordium II (Fig. 4). On the other hand, in the North American *P. weissei* and in *Paraurostyla coronata* ARORA *et al.*, 1999, there are two or three of such streaks, which are more-or-less parallel and apparently contribute to primordia II, III and IV [see Fig. 15 in JERKA-DZIADOSZ & FRANKEL (1969) and Figs 23, 24 in ARORA *et al.* (1999)]. JERKA-DZIADOSZ & FRANKEL (1969) also reported the formation of a “bare patch” of basal bodies in the space between the anterior region of the oral primordium and the proximal termini of the three leftmost FVT primordia of the North American *P. weissei*. Such structure is likely associated to agglomeration of basal bodies of the above mentioned streaks originating from the oral primordium. In *A. vernalis*, as only one of such streak develops, a proper patch is not formed.





Figs 9-14. Reorganizers after protargol-impregnation. Figs 9, 10. Ventral side of middle-to-late reorganizers. Notice the formation of surplus transverse cirri (black arrowheads) by primordia IV<sub>2</sub>, the leftmost forming also a second postperistomal cirrus (double black arrowhead). White asterisk shows vestigial procirri originating from primordia IV<sub>2</sub>, which are later resorbed; black asterisk shows late differentiation of adoral membranelles; black arrow shows differentiating undulating membranes; white arrow indicates residual right frontoventral cirrus; double white arrowhead shows outer dorsomarginal kinety originating from anterior end of right marginal primordium; and white arrowheads indicate newly formed dorsomarginal kineties. Figs 11-14. Nuclear apparatus of different specimens. Notice the dividing (white arrowheads) and non-dividing micronuclei (black arrowhead). Fronto-ventral-transverse primordia numbered in romans. Scale bar = 20  $\mu$ m.

Tab. I. Origins and products of the fronto-ventral-transverse primordia of *Apoamphisiella vernalis* (Stokes, 1887) Berger, 2006 during physiological reorganization.

Primordium	Origin	Product
I	Disaggregation of undulating membranes	Left frontal cirrus + undulating membranes
II	Disaggregation of buccal cirrus	Middle frontal cirrus + buccal cirrus + leftmost transverse cirrus
III	Disaggregation of left frontoventral cirrus + basal bodies from anterior end of oral primordium	Right frontal cirrus + left frontoventral cirrus + transverse cirrus
IV	Possibly associated to III + disaggregating cirri from anterior end of left ventral row	Right frontoventral cirrus + postperistomal cirrus + transverse cirrus
IV <sub>2</sub>	Possibly associated to IV + disaggregating cirri from anterior end towards the middle of left ventral row	Transverse cirrus, but sometimes, the leftmost anlage IV <sub>2</sub> produces also a second postperistomal cirrus.
V	Disaggregation of cirri slightly above equatorial region of right ventral row	Left ventral row + pretransverse cirrus + transverse cirrus
VI	Disaggregation of cirri slightly above equatorial region of right ventral row	Right ventral row + pretransverse cirrus + rightmost transverse cirrus

The short FVT primordia which arise between IV and V, herein named "IV<sub>2</sub>" are possibly related to IV, and may represent slightly modified replicas of it (DE CASTRO *et al.*, 2016). Evidence of such relationship comes from the primordia behavior, which is similar to IV, *viz.* forming transverse cirri, but not pretransverse; and sometimes forming a postperistomal cirrus (Fig. 10). Moreover, serial repetitions of primordium IV are common in hypotrichs. They are characteristic of the urostyloids, and probably evolved independently in patterniellids, neokeronopsids, and perhaps also in uroleptids and rigidothrichids (FOISSNER *et al.*, 2004; FOISSNER & STOECK, 2006, 2008; PAIVA *et al.*, 2009), forming the so-called midventral complex (BERGER, 2006). The pattern in which primordia IV<sub>2</sub> appear in early-

to-middle reorganizers, arranged in a ladderized sequence of short basal body streaks (Figs 7, 9), recall the corresponding developmental stage of a short patterniellid or *Territricha-Apoterritricha*-like midventral complex (FOISSNER, 1987; BERGER & FOISSNER, 1988; KIM *et al.*, 2014). Such structure, in *A. vernalis*, does not complete its development because the procirri which could mature into midventral pairs are resorbed before differentiation. Reorganization of the marginal cirral rows and dorsal kineties had no peculiarities, occurring as in most dorsomarginalians (e.g. GRIMES & ADLER, 1978; JERKA-DZIADOSZ & FRANKEL, 1969; KAMRA & SAPRA, 1991; ARORA *et al.*, 1999; BLATTERER & FOISSNER, 2003).

During physiological reorganization, the macronuclear nodules of *A. vernalis* approximate in middle reorganization, briefly touching each other and then to beginning to separate in middle-to-late reorganizers. Thus, complete fusion of the nodules was not observed. However, macronuclear fusion is reported in other dorsomarginalians (e.g. ARORA *et al.*, 1999; ZOU & NG, 1991; BLATTERER & FOISSNER, 2003), including the North American *P. weissei*, as described in JERKA-DZIADOSZ & FRANKEL (1969). Hence, we cannot exclude the possibility of a curt fusion being overlooked because the corresponding stage could be absent among the investigated specimens. In *A. vernalis*, not all micronuclei seemed to divide during physiological reorganization, since the studied reorganizers exhibited only one or two dividing micronuclei (among on average five of such structures). Conversely, in the North American *P. weissei*, the micronuclei divide to produce a total of eight units, indicating most or all micronuclei divide, even though subsequently from reorganization, this number is reduced to about four (JERKA-DZIADOSZ & FRANKEL, 1969).

The data on ontogeny during physiological reorganization confirmed the presence of dorsomarginal kineties, corroborating the classification of genus *Apoamphisiella* within the Dorsomarginalia Berger, 2006, as mentioned in PAIVA *et al.* (2014). Moreover, the ontogenetic similarities with the North American population of *P. weissei*, plus other morphological features, such as the cyrtohymenid pattern of the undulating membranes and the morphology and arrangement of cortical granules (GRIMES & L'HERNAULT, 1978; FOISSNER, 1997; PAIVA & SILVA-NETO, 2004; KÜPPERS & CLAPS, 2013), support the phylogenetic position of *Apoamphisiella* in the *Cyrtohymena-Paraurostyla* (BERGER, 1999; FOISSNER *et al.*, 2004) group of dorsomarginalian hypotrichs, which was recently hypothesized by DE CASTRO *et al.* (2016).

**Acknowledgements.** This study was financed by a master degree fellowship from CAPES granted to the first author through Programa de Pós-Graduação em Ecologia Aquática e Pesca – Universidade Federal do Pará (PPGEAP–UFPA), and funds from CNPq (Universal 485974/2013-4), FAPERJ (Biota E-26/110.022/2011) and FAPESP (2015/17856-0).

## REFERENCES

- ARORA, S.; GUPTA, R.; KAMRA, K. & SAPRA, G. R. 1999. Characterization of *Paraurostyla coronata* sp. n. including a comparative account of other members of the genus. *Acta Protozoologica* **38**:133-144.
- BERGER, H. 1999. Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). *Monographiae Biologicae* **78**:1-1080.
- BERGER, H. 2006. Monograph of the Urostyloidea (Ciliophora, Hypotricha). *Monographiae Biologicae* **85**:1-1304.
- BERGER, H. 2008. Monograph of the Amphiselliidae and Trachelostyloidea (Ciliophora, Hypotricha). *Monographiae Biologicae* **88**:1-737.
- BERGER, H. 2011. Monograph of the Gonostomatidae and Kahliliidae (Ciliophora, Hypotricha). *Monographiae Biologicae* **91**:1-741.
- BERGER, H. & FOISSNER, W. 1988. Revision of *Lamtostyla* Buitkamp, 1977 and description of *Territricha* nov. gen. (Ciliophora: Hypotrichida). *Zoologischer Anzeiger* **220**:113-134.
- BERGER, H. & FOISSNER, W. 1997. Cladistic relationships and generic characterization of oxytrichid hypotrichs (Protozoa, Ciliophora). *Archiv für Protistenkunde* **148**:125-155.
- BLATTERER, H. & FOISSNER, W. 2003. Morphological and ontogenetic comparison of two populations of *Parentocirrus hortualis* Voss 1997 (Ciliophora, Hypotrichida). *Linzer Biologische Beiträge* **35**:831-854.
- BORROR, A. C. 1979. Redefinition of the Urostylidae (Ciliophora, Hypotrichida) on the basis of morphogenetic characters. *Journal of Eukaryotic Microbiology* **26**:544-550.
- DA SILVA-NETO, I. D.; PAIVA, T. DA S.; DIAS, R. J. P.; CAMPOS, C. J. A. & MIGOTTO, A. E. 2012. Redescription of *Licnophora chattoni* Villeneuve-Brachon, 1939 (Ciliophora, Spirotrichea), associated with *Zyzyxuswarreni* Calder, 1988 (Cnidaria, Hydrozoa). *European Journal of Protistology* **48**:48-62.
- DE CASTRO, L. A. M.; KÜPPERS, G. C.; FERNANDES, N. M.; SCHLEGEL, M. & PAIVA, T. DA S. 2016. Ontogeny and molecular phylogeny of *Apoamphisiella vernalis* reveal unclear separation between genera *Apoamphisiella* and *Paraurostyla* (Protozoa, Ciliophora, Hypotricha). *PLoS One* **11**:e0155825.
- DEMBOWSKA, W. S. 1925. Studien fiber die Regeneration von *Stylonychia mytilus*. *Archiv für mikroskopische Anatomie und Entwicklungsmechanik* **104**:185-209.
- DEMBOWSKA, W. S. 1938. Körperreorganisation von *Stylonychia mytilus* beim Hungem. *Archiv für Protistenkunde* **91**:89-105.
- DEMBOWSKA, W. S. 1939. Sur la reorganisation du corps de *Stylonychia mytilus* sen inanition. *Comptes Rendus des Seances de la Societe de Biologie* **130**:565-567.
- DIECKMANN, J. 1995. An improved protargol impregnation for ciliates yielding reproducible results. *European Journal of Protistology* **31**:372-382.
- DRAGESCO, J. & DRAGESCO-KERNÉIS, A. 1986. Ciliés libres de l'Afrique intertropicale. Introduction à la connaissance et à l'étude des ciliés. *Faune tropicale* **26**:1-559.
- EIGNER, P. 1995. Divisional morphogenesis in *Deviata abbrevescens* nov. gen., nov. spec., *Neogeneia hortualis* nov. gen., nov. spec., and *Kahliliella simplex* (Horváth) Corliss and redefinition of the Kahliliidae (Ciliophora, Hypotrichida). *European Journal of Protistology* **31**:341-366.
- EIGNER, P. 1997. Evolution of morphogenetic processes in the Orthoamphisiellidae n. fam., Oxytrichidae, and Parakahliliidae n. fam., and their depiction using a computer method (Ciliophora, Hypotrichida). *Journal of Eukaryotic Microbiology* **44**:553-573.
- EIGNER, P. 1999. Comparison of divisional morphogenesis in four morphologically different clones of the genus *Gonostomum* and update of the natural hypotrich system (Ciliophora, Hypotrichida). *European Journal of Protistology* **35**:34-48.
- EIGNER, P. 2001. Divisional morphogenesis in *Uroleptus caudatus* (Stokes, 1886), and the relationship between the Urostylidae and the Parakahliliidae, Oxytrichidae, and Orthoamphisiellidae on the basis of morphogenetic processes (Ciliophora, Hypotrichida). *Journal of Eukaryotic Microbiology* **48**:70-79.
- EIGNER, P. & FOISSNER, W. 1992. Divisional morphogenesis in *Bakuella pampinaria* nov. spec. and reevaluation of the classification of the urostylids (Ciliophora, Hypotrichida). *European Journal of Protistology* **28**:460-470.
- EIGNER, P. & FOISSNER, W. 1993. Divisional morphogenesis in *Orthoamphisiella stramenticola* Eigner & Foissner, 1991 and *O. grelli* nov. spec. (Ciliophora, Hypotrichida). *Archiv für Protistenkunde* **143**:337-345.
- EIGNER, P. & FOISSNER, W. 1994. Divisional morphogenesis in *Amphisiellides illuvialis* n. sp., *Paramphisiella caudata* (Hemberger) and *Hemiamphisiella terricola* Foissner, and redefinition of the Amphisiellidae (Ciliophora, Hypotrichida). *Journal of Eukaryotic Microbiology* **41**:243-261.
- FLEURY, A. 1983. Exemple d'expression et de potentialites morphogenetiques chez des unicellulaires: le cas des hypotriches (protozoaires ciliés). *Annales de la Station Biologique de Besse-en-Chandesse* **17**:124-228.
- FOISSNER, W. 1987. Neue und wenigbekannte hypotriche und colpodide Ciliaten (Protozoa: Ciliophora) aus Böden und Moosen. *Zoologische Beiträge* **31**:187-282.
- FOISSNER, W. 1996. Ontogenesis in ciliated protozoa, with emphasis on stomatogenesis. In: HAUSMANN, K. & BRADBURY, P. C. eds. *Ciliates: Cells as organisms*. Stuttgart, Gustav Fischer, p. 95-177.

- FOISSNER, W. 1997. Soil ciliates (Protozoa: Ciliophora) from evergreen rain forests of Australia, South America and Costa Rica: diversity and description of new species. **Biology and Fertility of Soils** **25**:317-339.
- FOISSNER, W.; AGATHA, S. & BERGER, H. 2002. Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib Desert. **Denisia** **5**:1-1459.
- FOISSNER, W. & STOECK, T. 2006. *Rigidothrix goiseri* nov. gen., nov. spec. (Rigidotrichidae nov. fam.), a new "flagship" ciliate from the Niger floodplain breaks the flexibility-dogma in the classification of stichotrichine spirotrichs (Ciliophora, Spirotrichea). **European Journal of Protistology** **42**:249-267.
- FOISSNER, W. & STOECK, T. 2008. Morphology, ontogenesis and molecular phylogeny of *Neokeronopsis (Afrokeronopsis) aurea* nov.subgen., nov. spec. (Ciliophora: Hypotricha), a New African flagship ciliate confirms the CEUU hypothesis. **Acta Protozoologica** **47**:1-33.
- FOISSNER, W.; VAN DER STAAY, S. Y. M.; VAN DER STAAY, G. W. M.; HACKSTEIN, J. H. P.; KRAUTGARTNER, W. D. & BERGER, H. 2004. Reconciling classical and molecular phylogenies in the stichotrichines (Ciliophora, Spirotrichea), including new sequences from some rare species. **European Journal of Protistology** **40**:265-281.
- FOISSNER, W. & XU, K. 2006. Monograph of the Spathidiida (Ciliophora, Haptoria) Vol I: Protospathidiidae, Arcuospathidiidae, Apertospathulidae. **Monographiae Biologicae** **81**:1-490.
- FRONTCZAK-BANIEWICZ, M. & JERKA-DZIADOSZ, M. 1992. Conjugation-specific cortical reorganization during zygocyst formation in the hypotrich ciliate *Paraurostyla weissei*. **Acta Protozoologica** **31**:59-67.
- GRIMES, G. W. & ADLER, J. A. 1978. Regeneration of ciliary pattern in longitudinal fragments of the hypotrichous ciliate, *Stylonychia*. **Journal of Experimental Zoology** **204**:57-79.
- GRIMES, G. W. & L'HERNAULT, S. W. 1978. The structure and morphogenesis of the ventral ciliature in *Paraurostyla hymenophora*. **Journal of Protozoology** **25**:65-74.
- JERKA-DZIADOSZ, M. 1965. Morphogenesis of ciliature in division of *Urostyla weissei*. **Acta Protozoologica** **3**:345-353.
- JERKA-DZIADOSZ, M. 1972. Cortical development in *Urostyla*. I. Comparative study on morphogenesis in *U. cristata* and *U. grandis*. **Acta Protozoologica** **10**:73-100.
- JERKA-DZIADOSZ, M. & FRANKEL, J. 1969. An analysis of the formation of ciliary primordia in the hypotrich ciliate *Urostyla weissei*. **Journal of Protozoology** **16**:612-637.
- KAMRA, K. & SAPRA, G. R. 1991. Formation of a transient corticotype during excystment in *Coniculostomum monilata* (Oxytrichidae, Hypotrichida) and its modification by reorganizations. **European Journal of Protistology** **27**:331-342.
- KIM, J. H.; VD'ACNÝ, P.; SHAZIB, S. U. A. & SHIN, M. K. 2014. Morphology and molecular phylogeny of *Apoterritricha lutea* n. g., n. sp. (Ciliophora, Spirotrichea, Hypotrichia): a putative missing link connecting *Cyrtohymena* and *Afrokeronopsis*. **Journal of Eukaryotic Microbiology** **61**:520-536.
- KÜPPERS, G. C. & CLAPS, M. C. 2013. Hypotrichous ciliates (Protozoa: Ciliophora) from a temporary pond in Argentina, with redescription of *Apoamphisiella hymenophora* (Stokes, 1886) Berger, 1999. **Zootaxa** **3626**:55-76.
- LYNN, D. H. 2008. The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature, 3rd edn. Dordrecht, Springer. 608p.
- NG, S. F. 1990. Developmental heterochrony in ciliated protozoa: overlap of asexual and sexual cycles during conjugation. **Biological Reviews** **65**:19-101.
- PAIVA, T. DA S.; DIAS, R. J. P. & DA SILVA-NETO, I. D. 2014. Redescription of the little-known ciliate *Apoamphisiella vernalis* (Stokes, 1887) Berger, 2006 (Ciliophora, Hypotricha) from the Atlantic Forest, Brazil. **Journal of Eukaryotic Microbiology** **61**:561-568.
- PAIVA, T. DA S.; BORGES, B. DO N.; HARADA, M. L. & DA SILVA-NETO, I. D. 2009. Comparative phylogenetic study of Stichotrichia (Alveolata: Ciliophora: Spirotrichea) based on 18S-rDNA sequences. **Genetics and Molecular Research** **8**:223-246.
- PAIVA, T. DA S. & DA SILVA-NETO, I. D. 2004. Comparative morphometric study of three species of *Apoamphisiella* Foissner, 1997 (Ciliophora: Hypotricha) from Brazilian locations, including a description of *Apoamphisiella foissneri* sp. n. **Zootaxa** **505**:1-26.
- PAIVA, T. DA S. & DA SILVA-NETO, I. D. 2007. Morphology and morphogenesis of *Strongylidium pseudocrassum* Wang and Nie, 1935, with redefinition of *Strongylidium* Sterki, 1878 (Protista: Ciliophora: Stichotrichia). **Zootaxa** **1559**:31-57.
- PAIVA, T. DA S. & DA SILVA-NETO, I. D. 2009. Morphology and divisional morphogenesis of *Nudiamphisiella interrupta* Foissner, Agatha & Berger, 2002 (Ciliophora: Stichotrichia) based on a Brazilian strain. **European Journal of Protistology** **45**:271-280.
- SIQUEIRA-CASTRO, I. C. V.; PAIVA, T. DA S. & DA SILVA-NETO, I. D. 2009. Morphology of *Parastrongylidium estevesi* comb. nov. and *Deviata brasiliensis* sp. nov. (Ciliophora: Stichotrichia) from a sewage treatment plant in Rio de Janeiro, Brazil. **Zoologia** **26**:774-786.
- SMALL, E. B. & LYNN, D.H. 1985. Phylum Ciliophora Doflein, 1901. In: LEE, J. J.; HUTNER, S. H. & BOVEE, E. C. eds. **An illustrated guide to the Protozoa**. Lawrence, Society of Protozoologists, p. 393-575.
- STOKES, A. C. 1887. Some new hypotrichous infusoria from American fresh waters. **Annals and Magazine of Natural History** **20**:104-114.
- WALLENGREN, H. 1900. Studier öfver ciliate infusorier. IV. **Acta Universitatis Lundensis** **36**:1-54.
- WICKLOW, B. J. 1982. The Discocephalina (n. subord.): ultrastructure, morphogenesis and evolutionary implications of a group of endemic marine interstitial hypotrichs (Ciliophora, Protozoa). **Protistologica** **18**:299-330.
- WIRNSBERGER, E.; FOISSNER, W. & ADAM, H. 1985. Morphological biometric, and morphogenetic comparison of two close related species, *Stylonychia vorax* and *S. pustulata* (Ciliophora: Oxytrichidae). **Journal of Protozoology** **32**:261-268.
- ZOU, S. F. & NG, S. F. 1991. Physiological reorganization and post-traumatic regeneration in *Stylonychia mytilus*: reflections on developmental constraints and the evolutionary origin of alternative modes of asexual morphogenesis. **Journal of Protozoology** **38**:182-192.