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

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**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 cirilature 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 weissi* 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-
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RESULTS

Physiological reorganization in Apoamphisiella vernalis begins with the formation of an elongated, likely epiapokinetal, 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 anteriad 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 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...
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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 maturating 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, 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 primordium can maturate, becoming an extra postperistomal cirrus. Dorosomal kineties are formed right of right marginal row primordium, near its anterior end. The outer dorosomal kineties start its development contiguous 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
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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 primordium IV, Fig. 8. Dorsal side showing dorsal kinetics primordia (arrowheads). Fronto-ventral-transverse primordia numbered in romans. (IV, 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, 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.
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<table>
<thead>
<tr>
<th>Primordium</th>
<th>Origin</th>
<th>Product</th>
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<tbody>
<tr>
<td>I</td>
<td>Disaggregation of undulating membranes</td>
<td>Left frontal cirrus + undulating membranes</td>
</tr>
<tr>
<td>II</td>
<td>Disaggregation of buccal cirrus</td>
<td>Middle frontal cirrus + buccal cirrus + leftmost transverse cirrus</td>
</tr>
<tr>
<td>III</td>
<td>Disaggregation of left frontoventral cirrus + basal bodies from anterior end of oral primordium</td>
<td>Right frontal cirrus + left frontoventral cirrus + transverse cirrus</td>
</tr>
<tr>
<td>IV</td>
<td>Possibly associated to III + disaggregating cirri from anterior end of left ventral row</td>
<td>Right frontoventral cirrus + postperistomial cirrus + transverse cirrus</td>
</tr>
<tr>
<td>IV,</td>
<td>Possibly associated to IV + disaggregating cirri from anterior end towards the middle of left ventral row</td>
<td>Transverse cirrus, but sometimes, the leftmost anlage IV, produces also a second postperistomial cirrus.</td>
</tr>
<tr>
<td>V</td>
<td>Disaggregation of cirri slightly above equatorial region of right ventral row</td>
<td>Left ventral row + pretransverse cirrus + transverse cirrus</td>
</tr>
<tr>
<td>VI</td>
<td>Disaggregation of cirri slightly above equatorial region of right ventral row</td>
<td>Right ventral row + pretransverse cirrus + rightmost transverse cirrus</td>
</tr>
</tbody>
</table>

The short FVT primordia which arise between IV and V, herein named “IV,,” 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 postperistomial cirrus (Fig. 10). Moreover, serial repetitions of primordium IV are common in hypotrichs. They are characteristic of the urostyloids, and probably evolved independently in pattersoniellids, 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, 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 pattersoniellid 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).
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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 & FOISNNER, 2003), including the North American *P. weissei*, as described in JERKA-DZIADOSZ & FRANKEL (1969). Hence, we cannot exclude the possibility of a cut 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 kinetics, 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; FOISNNER, 1997; PAIVA & SILVA-NETO, 2004; KÜPPERS & CLAPS, 2013), support the phylogenetic position of *Apoamphisiella* in the *Cyrtohymena-Paraurostyla* (BERGER, 1999; FOISNNER et al., 2004) group of dorsomarginalian hypotrichs, which was recently hypothesized by DE CASTRO et al. (2016).

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