

Description of *Deviata rositae* n. sp., a New Ciliate Species (Ciliophora, Stichotrichia) from Argentina

GABRIELA C. KÜPPERS,^a ESTELA C. LOPRETTO^a and MARÍA C. CLAPS^b

^aCátedra Zoología Invertebrados I, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n, (1900) La Plata, Buenos Aires Province, Argentina, and

^bInstituto de Limnología “Dr. R. A. Ringuelet” (CONICET-UNLP), Av. Calchaquí Km 23,5, (1888) Florencio Varela, Buenos Aires Province, Argentina

ABSTRACT. Soil samples were taken from a temporary pond located in Buenos Aires province, Argentina, during the dry phase in the summer of 2005. The ciliates were studied alive and after staining with protargol. *Deviata rositae* n. sp. measures 112–154 µm in length and 21–28 µm in width in vivo and has a vermiform body. The contractile vacuole is located in the mid-body on the left. The macronucleus is moniliform and there are 1–3 micronuclei. The oral apparatus is composed of 14–18 adoral membranelles and straight paroral and endoral membranes that never intersect each other. The somatic ciliature is arranged in four frontal cirri, one buccal cirrus, six long and slightly spiraled rows of cirri with the first right row extending up to the equatorial or sub-equatorial region, and two dorsal rows of dikinetids. This new species of *Deviata* primarily differs from its congeners by the number of macronuclear nodules and the number and disposition of the dorsal rows of dikinetids.

Key Words. Ciliated protozoa, dry phase of a temporary pond, edaphic samples, morphology and infraciliature.

STUDIES of the ciliated fauna of Argentina using modern techniques have been mainly conducted in brackish water (Barría de Cao 2002; Pettigrosso 2001, 2003; Pettigrosso and Barría 2004; Pettigrosso, Barría de Cao, and Popovich 1997) and more recently in a freshwater temporary pond (Küppers, Lopretto, and Claps 2006a, b). Ciliates from soils constitute a completely unexplored field of investigation in this country, and with the exception of Foissner (1997), in South America as a whole. Temporary aquatic environments, such as puddles, pools, and small ponds, are very abundant in Buenos Aires province during the wet seasons. With the arrival of summer, most of them disappear and remain dry until the next wet season. In these habitats, the pond bed and the decomposing macrophytes that inhabited the pond become part of the terrestrial ecosystem during drought (Williams 1987). Formation of resting cysts by many ciliate species makes them well adapted to the changing conditions of these unstable habitats, and enables them to survive the complete loss of water from the environment (Foissner 1987). Therefore, transient habitats are ideal for the study of ciliates during both dry and aquatic phases.

The aim of this study is to describe a new species, *Deviata rositae* n. sp., isolated from soil samples taken during the dry phase of a temporary pond in Argentina.

MATERIALS AND METHODS

Soil samples were collected in January 2005 during the dry phase of a temporary pond located about 40 km south of the city of La Plata, Buenos Aires province, Argentina (35°05'S, 57°48'W). Six years of study of this pond showed that dry periods are mainly during the summer, and last 3–4 mo. The pond subsequently fills with rain water at the beginning of autumn. For a more detailed description of the study area, see Küppers et al. (2006a). The samples were taken from the first 5 cm of the soil and contained the dried sediment of the pond as well as dead macrophytes. In the laboratory, the samples were air-dried, stored at room temperature (~15–25 °C), and in January 2007, they were rewetted and treated with the non-flooded Petri dish method (Foissner 1987). Between 10 and 20 g of soil were placed in 15-cm Petri dishes. In some cases, distilled water was used to rewet the dried soil

material. In other cases, spring water with the addition of a wheat grain was added to enrich the medium, promoting bacterial growth and ciliate development. The ciliates were observed in vivo under the stereomicroscope and the light microscope at magnifications of 40X, 100X, and 400X. Fixation was made with Bouin's fluid and finally, the ciliates were stained with protargol following the protocol of Wilbert (1975). Measurements of impregnated cells were made at a magnification of 1,000X, with a calibrated ocular micrometer mounted on the light microscope. The illustrations were also made with the aid of a tracing device, at a magnification of 1,000X. The drawing of the living specimen is a free-hand sketch.

RESULTS

Description of *Deviata rositae* n. sp. (Table 1 and Fig. 1–7). The body size in vivo is 112–154 µm in length and 21–28 µm in width. It is vermiform in shape and round in cross-section, but the anterior end is slightly dorso-ventrally flattened. It is very flexible among soil particles. The cytoplasm is colorless, with 6–8 µm refractile granules that give it a grayish to blackish aspect at low magnification (<40X). Cortical granules are absent. The contractile vacuole is located in the mid-body on the left margin of the cell; it lacks collecting canals and empties dorsally. Some individuals possessed a defecation vacuole at the posterior end of the body. The food vacuoles contained 12–18 µm refractile structures, possibly wheat starch, and the ciliates also fed on bacteria (Fig. 1). The macronucleus is moniliform, with 7–14 spherical, ellipsoidal, or fusiform nodules. There are 1 to 3 ellipsoidal micronuclei and usually one of them is located near the first macronuclear nodule and the others are near the posterior nodules (Fig. 1–3, 6).

The oral apparatus is formed by 14–18 adoral membranelles of four rows of basal bodies each, and straight paroral and endoral membranes that never intersect each other. The paroral extends beyond the distal end of the endoral but it is proximally shorter. Both structures are composed of paired basal bodies. The oral zone represents 21% of the total length of the body (average of 20 impregnated cells) and cytopharyngeal fibers extend posteriorly from the cytostome to the right margin of the cell (Fig. 2, 4).

There are five cirri in the buccal field: three anterior-most frontal cirri, a fourth isolated frontal cirrus behind the right-most frontal one, and one buccal cirrus in front of the distal end of the endoral membrane. There are six slightly spiraled long rows of cirri that end

Corresponding Author: G. Küppers, Zoología Invertebrados I, Museo de La Plata, Paseo del Bosque s/n, (1900) La Plata, Buenos Aires province, Argentina—Telephone number: 54 221 4257744, ext. 132; FAX number: 54 221 4257527; e-mail: gkupperts@fcnym.unlp.edu.ar

Table 1. Morphometric data on *Deviata rositae* n. sp.

Character	X	M	Min.	Max.	SD	N
Body, length in vivo	128.3	126	112	154	12.3	15
Body, width in vivo	22.2	21	21	28	2.5	15
Body, length	129.5	126	98	154	13.7	20
Body, width	41.8	42	24.5	56	9.2	20
Macronucleus, number of nodules	8.9	8	7	14	2	20
Macronucleus, length of an anterior nodule	7.7	7.7	4.9	11.9	1.4	20
Macronucleus, width of an anterior nodule	5.6	5.4	4.2	9.1	1.3	20
Micronuclei, number	1.6	1.5	1	3	0.7	20
Micronucleus, length	3.5	3.5	2.8	4.5	0.4	20
Micronucleus, width	2.8	2.8	2.1	4.2	0.5	20
Adoral membranelles, number	17	17	14	18	1.1	20
Adoral zone of membranelles, length	26.8	28	21	35	3.1	20
Cytopharyngeal fibers, length	36.4	37.1	25.2	53.2	9.8	6
Anterior frontal cirri, number	3	3	3	3	0	20
Frontal cirri behind the right-most frontal, number	1	1	1	1	0	20
Buccal cirri, number	1	1	1	1	0	20
Long rows of cirri, total number	6	6	6	6	0	20
Long rows of cirri right of the adoral zone, number	3	3	3	3	0	20
Long rows of cirri left of the adoral zone, number	3	3	3	3	0	20
Cirri in the row 1, number	24.3	24	21	29	1.9	20
Cirri in the row 2, number	23.9	24	20	28	2	20
Cirri in the row 3, number	24.1	23.5	22	28	1.8	20
Cirri in the row 4, number	14	14	11	14	2	20
Cirri in the row 5, number	30.9	31	19	39	3.8	20
Cirri in the row 6, number	31.6	32.5	19	38	4.3	20
Cirri in lateral rows, length	12	12.6	9.8	14	1.1	15
Dorsal kineties, number	2	2	2	2	0	20
Basal body pairs in dorsal kinety 1, number	14	14	11	17	1.4	20
Basal body pairs in dorsal kinety 2, number	2.1	2	2	3	0.3	20
Dorsal bristles, length	3.6	3.5	2.4	4.2	0.6	15

All measurements are in μm . M, median; N, number of observations; SD, standard deviation; X, arithmetic mean; min. minimum value; max. maximum value.

Unless stated, measurements and countings were made on impregnated cells.

posteriorly of the adoral zone of membranelles. Three of them are located on the left of the adoral zone of membranelles (cirral rows 1–3), and the other three are located on the right of it (cirral rows 4–6) (Fig. 2, 3). Cirral rows 1 and 2 abut the adoral zone of membranelles on the left and extend to the posterior end on the dorsal surface of the body. Cirral row 3 extends from the post-peristomial region to the posterior end, with its distal segment sometimes on the dorsal surface. Cirral row 4 begins on the right just beneath the fourth frontal cirrus and extends up to the equator or sub-equator of the body (Fig. 2, 4). Cirral row 5 begins near the distal adoral membranelles and extends to the posterior end on the ventral surface. Cirral row 6 begins dorsally and also extends to the posterior end on the ventral surface. Anterior frontal cirri are formed by nine basal bodies each, while the frontal cirrus behind the right-most anterior frontal cirrus and the buccal cirrus possess six basal bodies each. The anterior-most cirri of rows 1–3 and 6 are composed of four basal bodies each, while the following cirri behind them have only two basal bodies. The anterior-most cirri of rows 4 and 5 are composed of six basal bodies, while some cirri behind them have four, and the following cirri behind the latter have only two basal bodies. Usually there is one pair (occasionally there are two pairs) of non-ciliated basal bodies, or even three very close basal bodies between cirral rows 3 and 4 (Fig. 2, 4, 5). Dorsally, there are two rows of bristles. The left-most row of bristles (dorsal kinety 1) extends from the anterior to the posterior end of the body and its posterior-most two dikinetids are slightly separated from the rest of the kinety, while the right-most row (dorsal kinety 2) is composed of only two dikinetids and is located at the anterior end of the body (Fig. 3, 7). Retained parental rows of cirri are absent, as well as transverse and caudal cirri.

The swimming behavior is in spirals, although they were observed motionless in the bottom of the Petri dish feeding on wheat starch and bacteria from the culture medium.

Occurrence. *Deviata rositae* n. sp. was isolated from dried soil samples collected in January 2005 and rewetted in January–February 2007. The species was found in row cultures of soil material together with another unidentified species of *Deviata*. This indicates that it developed from resting cysts, although these cysts were not observed.

DISCUSSION

This isolated ciliate was identified to the genus *Deviata* Eigner, 1995 because it has more than one long row of cirri on the left and right margins, of which one of the long rows of cirri right of adoral zone of membranelles extends up to the center of the ventral surface, and there are no remnant parental rows of cirri (Eigner 1995). Following Eigner (1995), these remnant parental cirri can be identified by the presence of additional rows of widely spaced and shorter cirri. In our isolate of *Deviata*, the number of cirral rows was constant at 6 ($N=20$), the cirri were equidistantly spaced, and the remaining rows were of about the same length, except for the row that extends up to the equator or sub-equator of the body. Therefore, retained parental cirri were absent. The diagnosis of the genus also states that these species develop multiple-within-anlagen; unfortunately, this could not be confirmed, since dividers were not found.

Comparison with related species. There are three species assigned to the genus *Deviata*, *Deviata abbrevescens* Eigner, 1995, the type species, *Deviata bacilliformis* (Gelei, 1954) Eigner, 1995, and *Deviata estevesi* Paiva and Silva-Neto, 2005. Our isolate

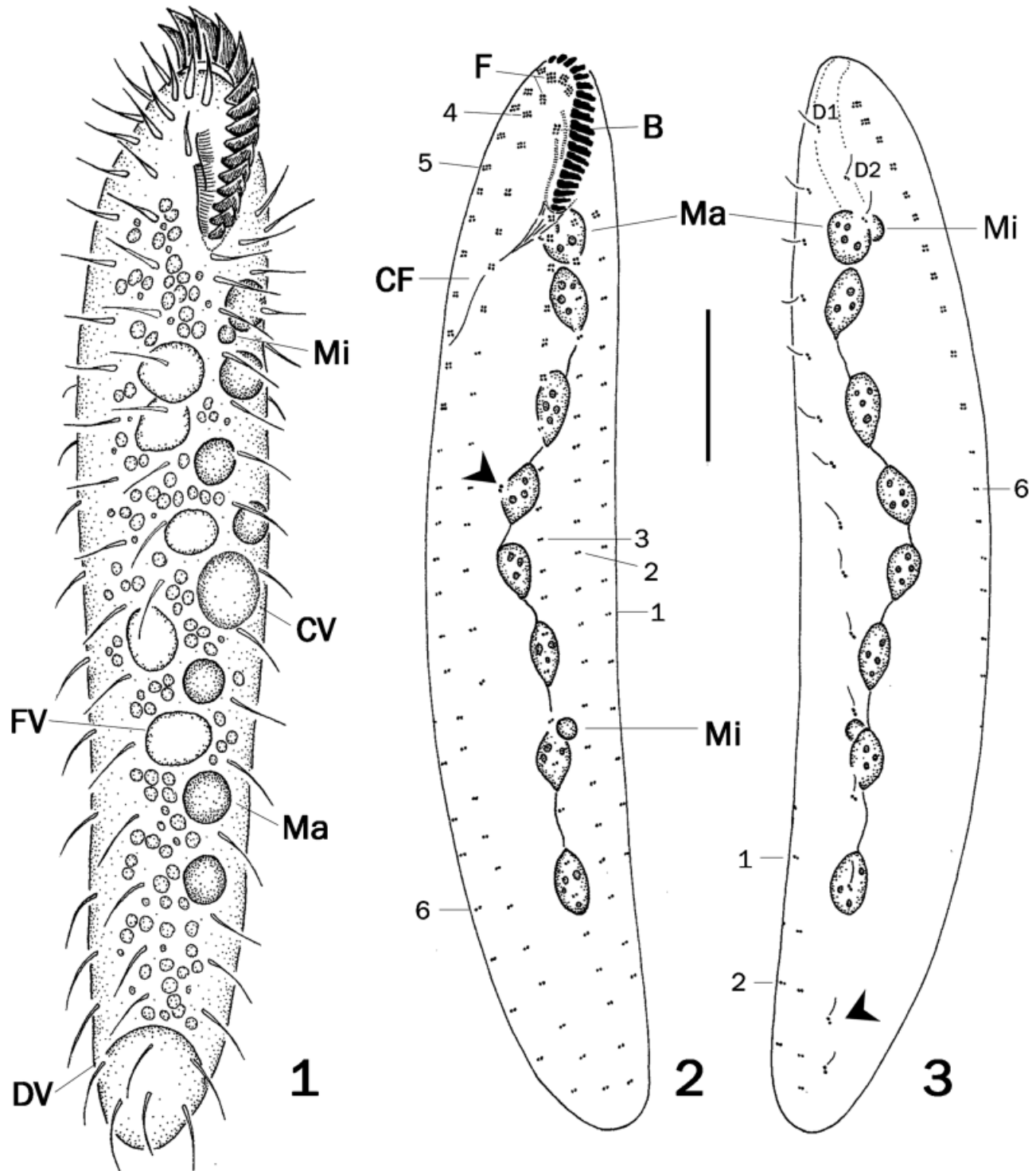


Fig. 1-3. *Deviata rositae* n. sp. after live observation (1) and protargol staining (2, 3). 1. Ventral view. 2. Ventral view. Pair of non-ciliated basal bodies (arrowhead). 3. Dorsal view showing dorsal kineties 1 (D1) and 2 (D2). Kinity 1 with separated posterior dikinetids (arrowhead). B, buccal cirrus; CF, cytopharyngeal fibers; CV, contractile vacuole; DV, defecation vacuole; F, frontal cirri; FV, food vacuole with wheat starch; Ma, macronucleus; Mi, micronucleus; 1-6, long rows of cirri. Scale bar = 20 μ m.

mainly differs from these three species by having a moniliform macronucleus and in the number and disposition of the dorsal rows of dikinetids. These characters are considered of taxonomic importance for the members of the family Kahliliidae (Berger and Foissner 1987). The new species we describe presents 7-14

macronuclear nodules, while there are two nodules in *D. abbrevescens*, two to four in *D. bacilliformis*, and two (rarely four) in *D. estevesi*. *Deviata bacilliformis* has only one dorsal kinety while our isolate, *D. abbrevescens*, and *D. estevesi* each have two dorsal kineties but with different dispositions. The right-most kinety

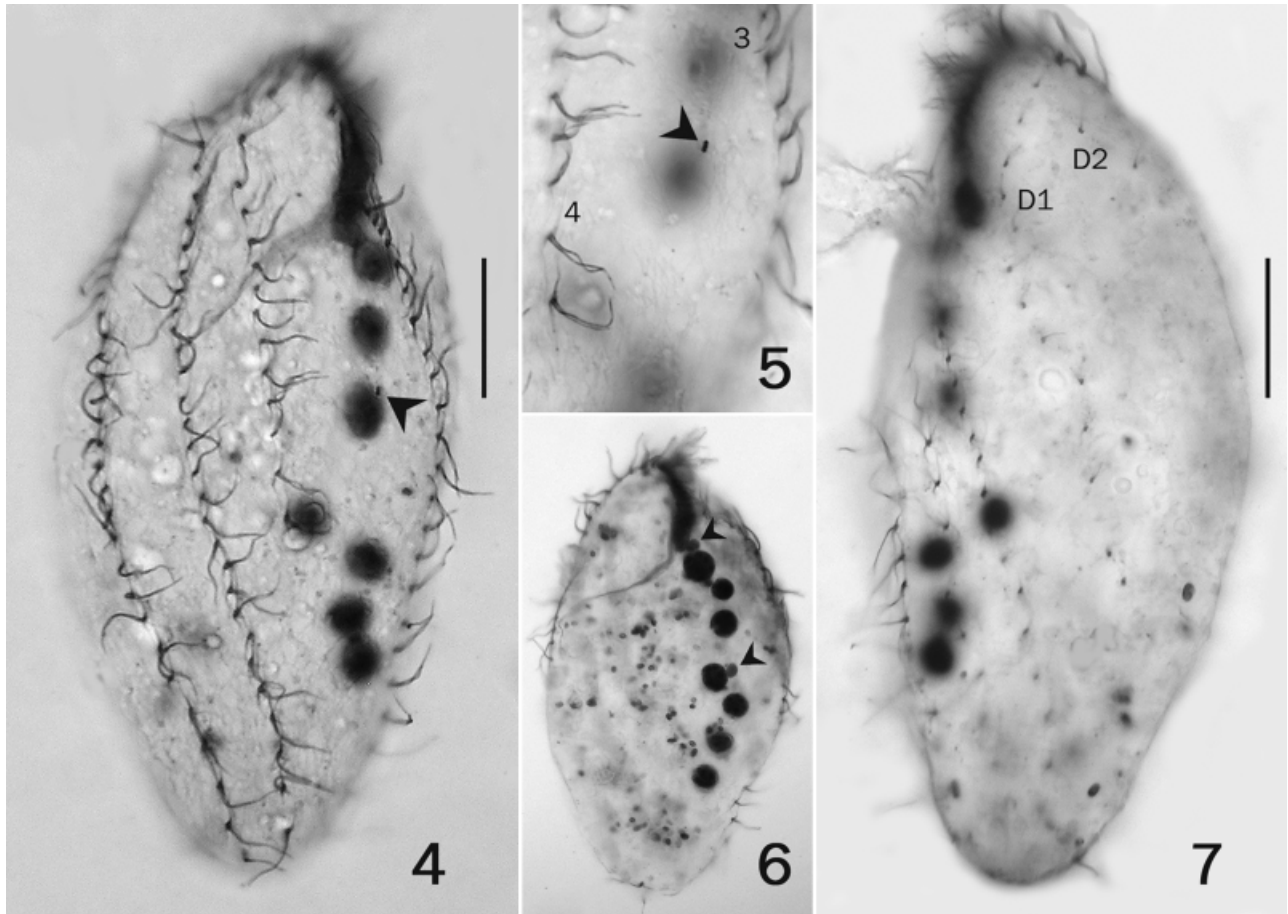


Fig. 4–7. *Deviata rositae* n. sp. after protargol staining. 4. Ventral view. Three very close non-ciliated basal bodies (arrowhead). 5. Higher magnification of non-ciliated basal bodies between cirral rows 3 and 4 (arrowhead). 6. Ventral view showing the nuclear apparatus. Micronuclei (arrowheads). 7. Dorsal view showing dorsal kineties 1 (D1) and 2 (D2). Scale bars = 20 μm .

in particular, has only a few dikinetids in our isolate. In *D. abbrevescens*, the right-most dorsal kinety extends from the anterior to the sub-terminal region of the body and presents 7–12 ($N = 20$) dikinetids, with the posterior-most ones widely spaced (Eigner 1995). In *D. estevesi*, the right-most dorsal kinety is interrupted in the equatorial zone and presents a shorter row of few dikinetids at the posterior end (3–5 dikinetids, $N = 5$; Paiva and Silva-Neto 2005). Another difference between our isolate and *D. estevesi* is the position of the contractile vacuole (in mid-body on the left vs. in mid-body centrally, respectively). While the contractile vacuole of *D. rositae* n. sp. lacks collecting canals, these are present in *D. bacilliformis*. The adoral zone of membranelles of our isolate is shorter (21% of total body length) and presents a lower number of oral polykinetids (14–18, $N = 20$) than *D. estevesi* (38%, calculated from the data of impregnated cells, and 28–33, $N = 22$, respectively; Paiva and Silva-Neto 2005). Between the known species of *Deviata* and our isolate, there are also differences in the number of long cirral rows. While our isolate has six long cirral rows, *D. abbrevescens* has seven (Eigner 1995), *D. bacilliformis* has 9–10 (Berger and Foissner 1987; Eigner 1995), and *D. estevesi* has nine (Paiva and Silva-Neto 2005).

The presence of non-ciliated basal bodies between cirral rows 3 and 4 in our isolate is a particular trait that has not been mentioned before for other congeners (Berger and Foissner 1987; Eigner 1995; Fleury and Fryd-Versafel 1984; Gelei 1954; Paiva and Silva-Neto 2005). These basal bodies are possibly involved in the

division morphogenesis. Thus, our isolate, named *D. rositae* n. sp., is clearly different from the other species in the genus *Deviata* and its status as a new species is justified. Further ontogenetic studies of *D. rositae* n. sp. are necessary to determine the origin of the non-ciliated basal bodies and confirm the presence of multiple-within-anlagen division morphogenesis.

Subclass Stichotrichia
Family Kahliellidae
Deviata rositae n. sp.

Diagnosis. Size in vivo 112–154 $\mu\text{m} \times 21$ –28 μm . Contractile vacuole without collecting canals, in mid-body on the left. Macronucleus moniliform, with 7–14 nodules. With 1–3 micronuclei. Adoral zone with 14–18 membranelles. With four frontal cirri, one buccal cirrus, six long and slightly spiraled rows of cirri, and two dorsal rows of dikinetids. Right-most dorsal kinety composed of few dikinetids, at the anterior end of the body.

Type locality. In soil collected during the dry phase from a temporary freshwater pond (35°05'S, 57°48'W), located near the city of La Plata, Buenos Aires province, Argentina.

Dedication. This species is dedicated to Dr. Rosa E. Pettigrosso, after her nickname “Rosita.” Dr. Pettigrosso is a pioneer in the study of ciliates from Argentina with modern techniques, and she kindly shared her knowledge with us.

Type slides. The type slide of *D. rositae* n. sp. was deposited as one protargol stained slide in the Colección de Invertebrados of the Museo de La Plata, La Plata, Buenos Aires Province, Argentina (Accession number MLP029), with relevant cells marked. A paratype slide was also deposited in the Ciliate Type Slide Collection, Smithsonian Institution, Washington, USA (Accession number USNM11106151).

ACKNOWLEDGMENTS

We are thankful to Dr. Lía Lunaschi and Dr. Fabiana Drago from the División de Invertebrados from the Museo de La Plata for allowing us to use the camera lucida, and to Santiago Nenda for his help with the photographs. Support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) is also greatly acknowledged.

LITERATURE CITED

- Barría de Cao, M. S. 2002. Citología, Bioecología y Taxonomía de Tintinnida (Ciliophora). Dissertation, Universidad Nacional del Sur, Bahía Blanca, Argentina. 213 p.
- Berger, H. & Foissner, W. 1987. Morphology and biometry of some soil hypotrichs (Protozoa: Ciliophora). *Zool. Jb. Syst.*, **114**:193–239.
- Eigner, P. 1995. Divisional morphogenesis in *Deviata abbrevescens* nov. gen., nov. spec., *Neogeneia hortualis* nov. gen., nov. spec., and *Kahliella simplex* (Horváth) Corliss and redefinition of the Kahliellidae (Ciliophora, Hypotrichida). *Eur. J. Protistol.*, **31**:341–366.
- Fleury, A. & Fryd-Versafel, G. 1984. Unité et diversité chez les hypotriches (Protozoaires Ciliés). I—Approche morphogénétique par l'étude de quelques formes peu différenciées. *Protistologica*, **20**:525–546.
- Foissner, W. 1987. Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. *Prog. Protistol.*, **2**:69–212.
- Foissner, W. 1997. Soil ciliates (Protozoa: Ciliophora) from evergreen rain forests of Australia, South America and Costa Rica: diversity and description of new species. *Biol. Fertil. Soils*, **25**:317–339.
- Gelei, J. 1954. Über die Lebensgemeinschaft einiger temporärer Tümpel auf einer Bergwiese im Börzsönygebirge (Oberungarn) III. Ciliaten. *Acta Biol. Hung.*, **5**:259–343.
- Küppers, G. C., Lopretto, E. C. & Claps, M. C. 2006a. Morphological aspects and seasonal changes of some planktonic ciliates (Protozoa) from a temporary pond in Buenos Aires Province, Argentina. *PANAMJAS*, **1**:74–90.
- Küppers, G. C., Lopretto, E. C. & Claps, M. C. 2006b. *Pelagostrobilidium wilberti* n. sp. (Oligotrichea, Choreotrichida): morphology and morphogenesis. *J. Eukaryot. Microbiol.*, **53**:477–484.
- Paiva, T. S. & Silva-Neto, I. D. da. 2005. *Deviata estevesi* sp. n. (Ciliophora: Spirotrichea), a new ciliate protist from a restinga lagoon in Rio de Janeiro, Brazil. *Acta Protozool.*, **44**:351–362.
- Pettigrosso, R. E. 2001. Estudio Taxonómico y Ecológico de Ciliados Planctónicos (Ciliophora: Choreotrichida y Strombidiida) del Estuario de Bahía Blanca, Argentina. Dissertation. Universidad Nacional del Sur, Bahía Blanca, Argentina, 173 p.
- Pettigrosso, R. E. 2003. Planktonic ciliates Choreotrichida and Strombidiida from the inner zone of Bahía Blanca estuary, Argentina. *Iheringia (Ser. Zool.)*, **93**:117–126.
- Pettigrosso, R. E. & Barría, M. S. 2004. Ciliados planctónicos. In: Piccolo, M. C. & Hoffmeyer, M. S. (ed.), Ecosistema del Estuario de Bahía Blanca. IADO, Bahía Blanca, Argentina. p. 121–131.
- Pettigrosso, R. E., Barría de Cao, M. S. & Popovich, C. A. 1997. Planktonic ciliates during a diatom bloom in Bahía Blanca Estuary, Argentina. I Aloricate. *Oebalia*, **23**:21–31.
- Wilbert, N. 1975. Eine verbesserte Technik der Protargolimprägung für Ciliaten. *Mikrokosmos*, **64**:171–179.
- Williams, D. D. 1987. The Ecology of Temporary Waters. Timber Press, Portland, OR. p. 3.

Received: 06/04/07, 07/18/07; accepted: 07/19/07