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Description of *Notohymena pampasica* n. sp. (Ciliophora, Stichotrichia)

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Summary. *Notohymena pampasica* n. sp. was isolated from terrestrial samples obtained from a temporary pond during its dry phase in Buenos Aires province, Argentina. The ciliates were grown in resuspended soil material and were studied *in vivo* and after impregnation with protargol. The species measures 84–112 × 35–42 μm *in vivo*. The organisms'cytoplasm is transparent, with many refractive cytoplasmic globules, which appear dark brown at low magnification, and are located mainly at the posterior end and the margins of the body. There are colorless refractive cortical granules in longitudinal rows at the bases of dorsal dikinetids and around ventral cirri. The contractile vacuole presents two inconspicuous collecting canals. The nuclear apparatus is composed of 2 macronuclear nodules and 2 (rarely 3) micronuclei. There are 22–29 oral polykinetids and paroral and endoral membranes arranged in a pattern typical of *Notohymena*. The somatic ciliature is arranged in 3 frontal, 1 buccal, 4 fronto-ventral, 3 (rarely 4) postoral, 2 pre-transverse, 5 transverse, and 2 marginal rows of cirri. Dorsally there are 6 rows of dikinetids and 3 caudal cirri. This new species primarily differs from congeners in the color and pattern of disposition of the cortical granules.

Key words: *Notohymena pampasica* n. sp., morphology, infraciliature.

INTRODUCTION

The species described within the genus *Notohymena* Blatterer and Foissner, 1988 are mostly soil inhabitants that have been found on four continents. *Notohymena rubescens* Blatterer and Foissner, 1988 was discovered in soil samples with lichens and mosses from Australia (Blatterer and Foissner 1988) and in mosses and soil samples from Germany and France (Voss 1991). *Notohymena antarctica* Foissner, 1996 was first found in a grass sward from Antarctica and later in soils from Scotland (Esteban *et al.* 2001, 2006) and soils from Austria (Foissner *et al.* 2005). *Notohymena australis* (Foissner and O'Donoghue, 1990) Berger, 1999, on the other hand, was isolated from limnetic environments, such as a small pond from Australia (Foissner and O'Donoghue 1990) and the Amper River in Germany (Foissner and Gschwind 1998). *Notohymena selvatica* (Hemberger, 1985) Blatterer and Foissner, 1988 is the only species known from South America and was found in woodland soils from Perú.

Many ciliate species were described or redescribed after the work of Kahl (1930–1935) was published. The number of extant, free-living ciliates is estimated

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to be close to 3000 (Finlay *et al.* 1996). According to Foissner however, this number may be close to 30 000 species, considering that ciliate diversity is still largely undescribed (Foissner 2006). Most taxonomic studies on ciliates were carried out in Europe, leaving the ciliated fauna from South America, particularly terrestrial ciliates, almost unexplored.

The aim of the present study is to describe the morphology of a new species, *Notohymena pampasica* n. sp., which was isolated from the dry sediment of a temporary pond in Argentina. It was studied using live observations and silver staining with protargol.

MATERIALS AND METHODS

The sampling site is a temporary pond located in Buenos Aires province, Argentina (35°05′S, 57°48′W) (Fig. 1). Samples were taken during the years 2003–2005. The pond goes through drought periods several times during the annual cycle, mainly in the summer. Soil samples were taken that contained sediment as well as dead macrophytes from the pond during these dry phases. Raw cultures, kept at room temperature (15–25°C), were made following the Petri dish method (Foissner 1987) from air-dried soil material and a wheat grain, which was added to rewetted samples in order to enrich the medium. The ciliates were observed *in vivo* and after impregnation with protargol (Wilbert 1975). Cyst formation was encouraged by placing some living individuals on a slide with a coverslip with vaseline at the corners. These slides were left in a wet chamber and were inspected in the days following in order to verify the encystment. Observations were made at magnifications of 40 ×, 100 ×, 400 ×, and 1000 × under a bright field microscope. The drawings of living specimens were based on free-hand sketches. Drawings of impregnated cells were made with the aid of a tracing device, at 1000 ×. Photographs were taken at magnifications of 400 × and 1000 ×. The terminology used is according to Berger (1999) and Foissner and Al-Rasheid (2006).

RESULTS

Notohymena pampasica n. sp. (Figs 2, 4–17; Table 1)

Diagnosis: Body size *in vivo* of 84–112 × 35–42 µm. Cytoplasm transparent, with refractive globules that appear dark brown at low magnification. Colorless refractive cortical granules occur in longitudinal rows at the bases of dorsal dikinetids and around ventral cirri. Adoral zone with 22–29 membranelles. Right marginal row with 15–21 cirri, left marginal row with 14–21 cirri. With 5 transverse cirri, 6 dorsal rows of dikinetids, and 3 caudal cirri.

Type location: Soil samples from a temporary pond in Buenos Aires province, Argentina (35°05′S, 57°48′W), during its dry phase.

Type material: Protargol slides, with the relevant organisms marked with black ink, have been deposited in the Invertebrates Collection of the Museo de La Plata (accession numbers MLP 027, MLP 028), La Plata, Argentina.

Etymology: Named after the pampasic region in Argentina where the species was found.

Description: Size *in vivo* of 84–112 × 35–42 µm. The body is elliptical, dorso-ventrally flattened, slightly contractile, and very flexible when moving among detritus particles. The cytoplasm is transparent, filled with 1–4 µm (N = 20) *in vivo* across refractive globules that appear dark brown at low magnification (less than 100 ×). These globules are more densely packed mainly at the posterior end and the cell margins (Figs 2, 7–10), but they are also present at the mid-body. There are colorless, refractive cortical granules that are mainly distributed following the dorsal somatic ciliature (impregnated, sometimes faintly, with protargol). These granules (0.7–1 µm in width *in vivo*, N = 20) are arranged dorsally in longitudinal rows at the bases of dikinetids and ventrally, mainly around cirral bases. Some irregular groups of cortical granules were also observed among dorsal kineties (Figs 6, 14–16). The contractile vacuole presents two inconspicuous collecting canals during diastole. It is located almost in mid-body toward the left margin, and empties dorsally (Figs 2, 8, 10). Fecal balls were released dorsally at the posterior

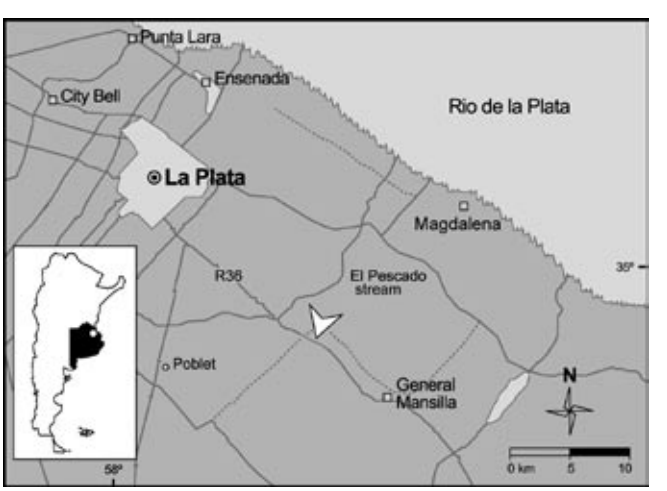
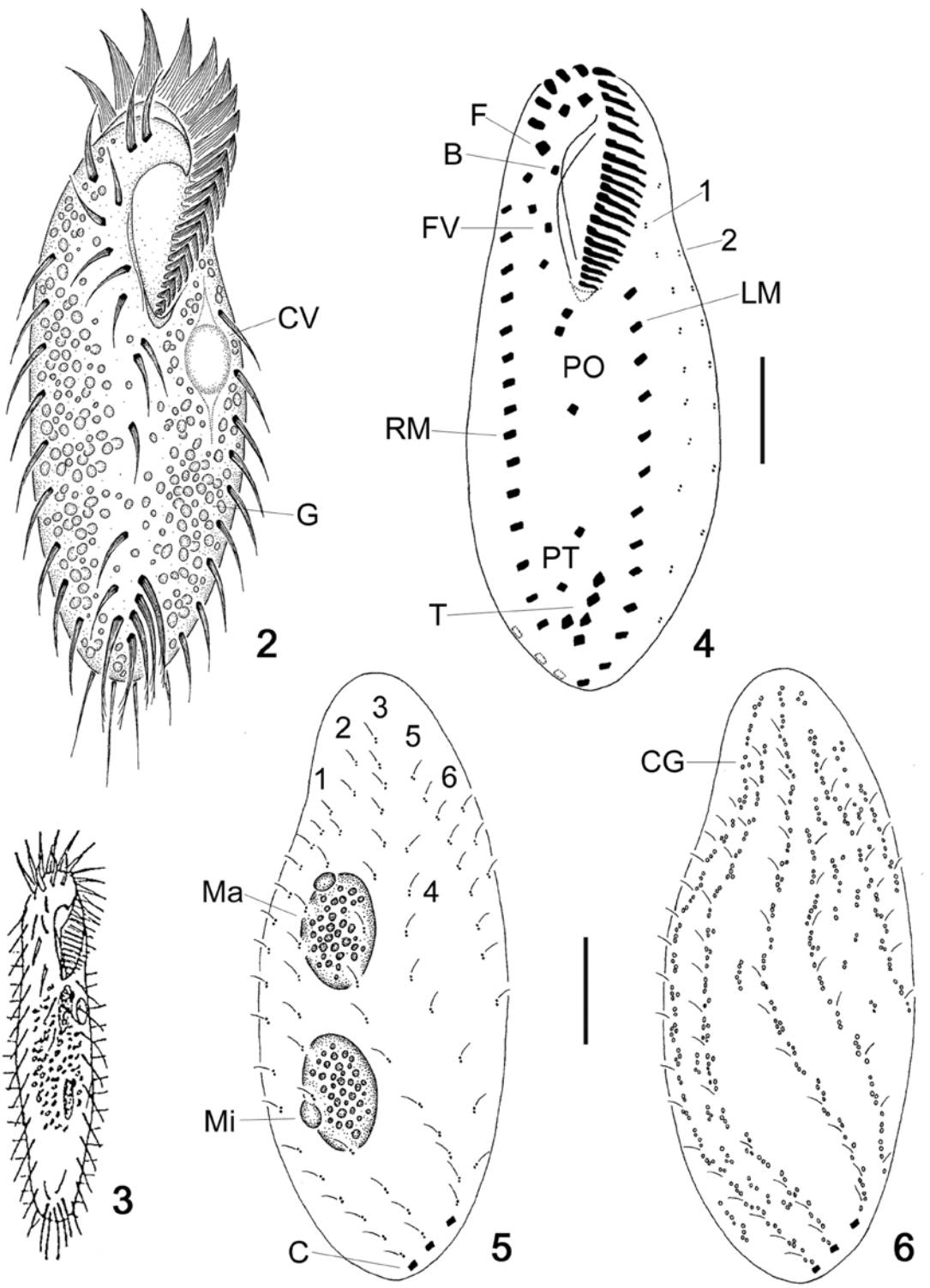


Fig. 1. Map showing the location of the sampling site in Buenos Aires province, Argentina.

Table 1. Morphometric data of *Notohymena pampasica* n. sp. Unless indicated, data based on protargol-impregnated specimens. All measurements in μm. M – median, Max – maximum, Min – minimum, N – number of observations, SD – standard deviation, $\overline{x}$ – arithmetic mean.

Character	$\overline{x}$	M	Min	Max	SD	N
Body length, *in vivo*	96.4	98	84	112	10.9	9
Body width, *in vivo*	38.8	42	35	42	3.7	9
Cysts diameter, *in vivo*	45	45.1	38.9	49.2	3.2	10
Body length	83.8	82.6	71.4	100.8	7.9	20
Body width	35.3	33.6	27.3	46.2	5.5	20
Adoral zone of membranelles, length	32.3	32.2	28	36.4	2.4	20
Distance from posterior postoral to anterior-most pre-transverse cirrus	25.3	25.2	18.2	42	6.1	20
Distance from posterior somatic end to posterior-most transverse cirrus	4.2	4.2	2.8	5.6	1	20
Distance between macronuclear nodules	7.7	7	4.2	11.9	2.3	20
Macronuclear nodules, number	2	2	2	2	0	20
Macronucleus, length	13.3	13.3	11.2	16.1	1.5	20
Macronucleus, width	9.7	9.8	7	12.6	1.5	20
Micronucleus, number	2.1	2	2	3	0.3	20
Micronucleus, length	3.2	2.9	2.8	4.2	0.5	20
Micronucleus, width	2.8	2.8	2.4	3.5	0.3	20
Membranelles, number	25.7	26	22	29	1.5	20
Frontal cirri, number	3	3	3	3	0	20
Fronto-ventral cirri, number	4	4	4	4	0	20
Postoral ventral cirri, number	3.1	3	3	4	0.3	20
Pre-transverse ventral cirri, number	2	2	2	2	0	20
Transverse cirri, number	5	5	5	5	0	20
Buccal cirri, number	1	1	1	1	0	20
Right marginal cirri, number	17.4	17	15	21	1.3	20
Left marginal cirri, number	17.1	17	14	21	1.8	20
Dorsal kineties, number	6	6	6	6	0	20
Caudal cirri, number	3	3	3	3	0	20

end of the body (Fig. 9). The nuclear apparatus is composed of 2 ellipsoidal macronuclear nodules located at the left of the body mid-line, and 2 (rarely 3) ellipsoidal micronuclei (Figs 5, 15). There are 22–29 adoral membranelles and the paroral and endoral membranes have the same pattern typically seen in *Notohymena*. The paroral membrane has a hooked distal end and kinetosomes arranged in a zig-zag. The endoral membrane has double kinetosomes in a simple row (Figs 3, 13, 17). The adoral zone of membranelles does not reach the mid-body (reaching about 38% of the body length, on average after protargol). The frontal membranelles



Figs 2–6. Illustration of *Notohymena pampasica* n. sp. from life (2) and after protargol impregnation (4–6), and comparison with *Cyrtohymena granulata* (3). **2** – ventral view. CV – contractile vacuole; G – cytoplasmic globules. **3** – *Cyrtohymena granulata in vivo* (from Kahl 1932). **4** – ventral infraciliature. B – buccal cirrus; F – frontal cirri; FV – fronto-ventral cirri; LM – left marginal cirri; PO – postoral cirri; PT – pre-transverse cirri; RM – right marginal cirri; T – transverse cirri; 1, 2 – dorsal kineties 1 and 2. **5** – dorsal ciliature and nuclear apparatus. C – caudal cirri; Ma – macronucleus; Mi – micronucleus; 1–6 – dorsal kineties 1 to 6. **6** – dorsal view showing cortical granules (CG). Scale bars: 20 μm.

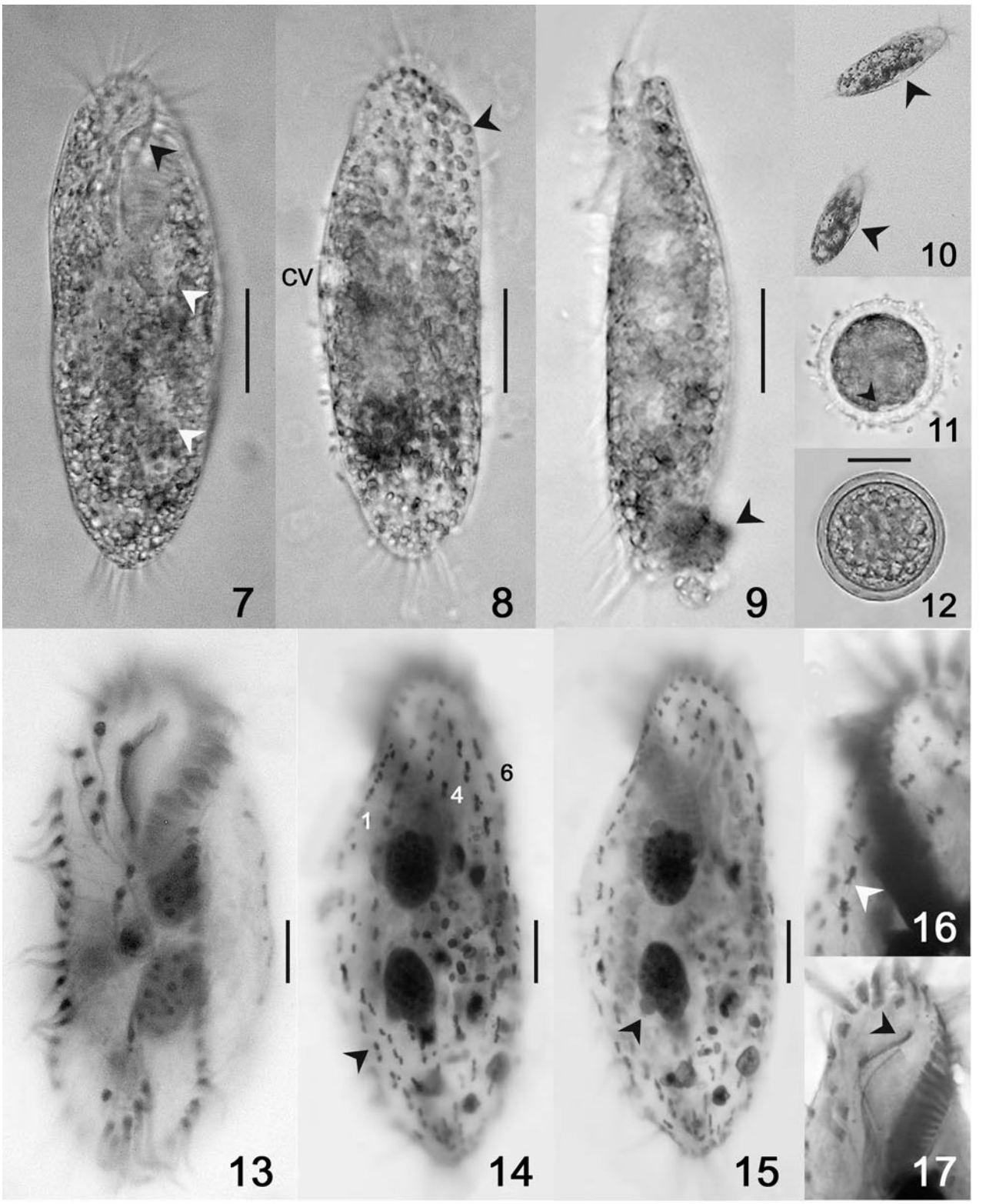
are 16.4–20.5 µm (N = 7) long *in vivo*. There are inconspicuous lateral membranellar cilia *ca* 3 µm long arising from ventral membranelles. Membranellar bolsters were not distinguished. The buccal lip is curved and there is a buccal horn (Figs 2, 7). The paroral membrane runs along the edge of the buccal lip. The ventral somatic ciliature is composed of 18 fronto-ventral-transverse cirri arranged in typical groups. There are 3 strong frontal cirri, 1 buccal cirrus near the intersection of the paroral and endoral membranes, 4 fronto-ventral cirri, 3 (rarely 4) postoral cirri, 2 pre-transverse cirri, 5 transverse cirri, 15–21 right marginal cirri, and 14–21 left marginal cirri. Transverse cirri (12.3–18.4 µm long *in vivo*, N = 7) are arranged in a V pattern that extend beyond the posterior end of the cell, and have frayed ends. Marginal cirri are 12.3 µm (N = 7) long *in vivo*; parallel right and left rows are interrupted posteriorly, with the left marginal row running continuously with the dorsal caudal cirri (Figs 3, 13).

The dorsal somatic ciliature is composed of 6 rows of dikinetids, with the most anterior kinetosome bearing a 3.5–4.5 µm long bristle. Dorsal kinety 1 is shortened anteriorly and extends to the posterior end of the body. Dorsal kineties 2, 3, and 5 extend from the anterior to the posterior end, with kinety 5 being slightly shortened anteriorly. Dorsal kinety 4 begins in the middle third, is conspicuously shortened anteriorly, and reaches the posterior end of the body. Dorsal kinety 6 is formed from 2–3 dikinetids (N = 15) that extend along the anterior third of the body. There are 3 caudal cirri displaced to the posterior margin of the body at the ends of dorsal kineties 1, 2, and 4 (Figs 5, 14, 16). Caudal cirri are approximately as long as transverse cirri (16.4–20.5 µm long *in vivo*, N = 7). The cysts average 45 µm across *in vivo*, with smooth walls, and granulated content. At an earlier stage of encystment there is an inconspicuous wall surrounding the rounded organism. Next, the wall becomes thicker (*ca* 2.5 µm in thickness) and the contractile vacuole still pulsates at this stage (Fig. 11). Later, the cyst has a smooth wall *ca* 7 µm in thickness (Fig. 12).

Occurrence: *Notohymena pampasica* n. sp. was recorded in soil samples obtained in January 2004. It was resuspended with distilled water and a wheat grain in August 2006–January 2007. It was found to be very abundant in the cultures and persistent for *ca* two-three weeks.

DISCUSSION

The genus *Notohymena* was erected for those species with arched undulating membranes crossing behind the buccal cirrus, and a paroral membrane with a hooked distal end (Blatterer and Foissner 1988). Until the present finding, there were four species described within this genus. *Notohymena pampasica* n. sp. primarily differs from these species in the color of the cortical granules, the pattern of disposition of these cortical granules and, in one case, the number of caudal cirri. *Notohymena pampasica* n. sp. is smaller in size than *N. selvatica* and presents 5, instead of 4, transverse cirri (Hemberger 1985). Hemberger did not observe the cortical granules of *N. selvatica* (if present). Unlike *N. pampasica* n. sp., *N. rubescens* presents ruby-red cortical granules (Blatterer and Foissner 1988). *Notohymena pampasica* n. sp. not only differs from *N. antarctica* in the color of the cortical granules (colorless *vs.* yellow to yellow-green, respectively) but also in the dorsal disposition of these granules (longitudinal rows at the bases of dorsal dikinetids *vs.* small groups around dorsal dikinetids, respectively) (Foissner 1996). The number of oral polykinetids is slightly lower in *N. pampasica* n. sp. than in *N. antarctica* (26 *vs.* 30, mean values, respectively). In both species, the left marginal cirri are continuous with the dorsal caudal cirri. The cortical granules of *N. pampasica* n. sp. have a similar pattern of disposition on the dorsal surface as *N. australis*, but the color of these granules differs (colorless *vs.* yellow-green to orange-green, respectively) (Foissner and O'Donoghue 1990, Foissner and Gschwind 1998, Berger 1999). These species also differ in the number of micronuclei (2, rarely 3 *vs.* 1–4, usually 3, respectively), the variability of the number of postoral, pre-transverse, and transverse cirri (in *N. australis*), and the number and disposition of caudal cirri (invariably 3 *vs.* 6–11, respectively) (Foissner and O'Donoghue 1990, Foissner and Gschwind 1998). A particular trait observed in *N. pampasica* n. sp., was the presence of refractive cytoplasmic globules that appear dark at low magnification. This characteristic is shared with *Cyrtohymena granulata* (Kahl 1932) Foissner 1989, which is filled with dark, sometimes green, granules in its cytoplasm (Kahl 1932) (Fig. 3). However, more differences than similarities exist between *N. pampasica* n. sp. and *C. granulata*. The most important one, which was unmentioned by Kahl (1932), is the presence of cortical granules, which are so conspicuous in related species. *Notohymena pampasica* n. sp. has a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different proportion of body length to body width (2:1 *vs.* 4:2, respectively), a lower distance between the posterior-most postoral and the anterior-most pre-transverse ventral cirri (inferred from Kahl's drawing), apparently shorter dorsal bristles, and the contractile vacuole presents collecting canals during diastole. Nevertheless, the lack of reference material to compare Kahl's species with ours still makes the identification uncertain. On the other hand, since the cortical granules of the species we studied are colorless, Kahl could have overlooked them. Further research might show whether *Notohymena pampasica* n. sp. is indeed a new species or if it should be a new genus combination for *C. granulata*.

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Figs 7–17. Micrographs of *Notohymena pampasica* n. sp. from life (7–12) and after protargol impregnation (13–17). **7** – ventral view showing the buccal horn (black arrowhead) and macronuclear nodules (white arrowheads). **8** – dorsal view showing cytoplasmic globules (arrowhead) and contractile vacuole (CV). **9** – lateral view showing a fecal ball at the posterior end of the body. **10** – ventral view at lower magnification (100 ×), contractile vacuole with collecting canals (arrowheads). **11** – resting cyst, early stage, formation of contractile vacuole (arrowhead). **12** – resting cyst, late stage. **13** – ventral view. **14** – dorsal view showing cortical granules (arrowhead). 1, 4, 6 – longitudinal rows of cortical granules at the bases of dorsal kineties 1, 4, and 6. **15** – dorsal view showing the nuclear apparatus. Arrowhead points micronucleus. **16** – dorsal view, magnification of cortical granules at the base of dorsal dikinetids (arrowhead). **17** – ventral view, magnification of the oral apparatus. Arrowhead points the hooked end of paroral kinety. Scale bars: 20 μm (7–9, 11, 12), 10 μm (13–15).