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Cytogenetic analysis of four argentinean populations of *Artemia* (Crustacea Branchipoda: Anostraca)

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Abstract — *Artemia* brine shrimps inhabit hypersaline ecosystems all over the world except Antarctica. In South America two bisexual diploid species are encountered: *Artemia franciscana* Kellogg distributed along America and *Artemia persimilis* Piccinelli and Prosdocimi restricted to Argentina and a few Chilean localities. *A. franciscana* was recently cited for the first time in our country and our results suggested a possible hybridization in Las Tunas Lagoon (Córdoba province) between both species. In the present work four new Argentinean populations of *Artemia* have been cytogenetically analyzed by conventional staining, silver staining, and C and DAPI banding. The populations Salina La Antigua (La Rioja province), Salinas Grandes (Córdoba province) and Pampa de las Salinas (San Luis province) gave similar results compatible with *A. franciscana* (2n=42, n=21). Conversely, Salitral de la Vidriera population (Buenos Aires province) would correspond to *A. persimilis* (2n=44). While in Salitral de la Vidriera only one or two small chromocenters C positive and DAPI bright were detected, in Pampa de las Salinas and Salinas Grandes many of them were observed. Nevertheless, both populations presented less heterochromatin than the *Artemia franciscana* reference strain from Great Salt Lake (USA). These results constitute further evidence to the occurrence of heterochromatin variations not only between both species but also within each one. Last but not least, with the present contribution *A. franciscana* is now cited in three new Argentinean localities.

Key words: C-banding, fluorescent banding, heterochromatin, meiosis, mitosis

INTRODUCTION

Crustaceans of the genus *Artemia* inhabit hypersaline environments and they are distributed from the sea level up to 4500 m altitude under very different climates in all continents except Antarctica (VANHAECKE *et al.* 1987; TRIANTAPHYLLIDIS *et al.* 1998; VAN STAPPEN 2002). In the last 20 years *Artemia* has been the object of both basic and applied research; it has become an ideal model for diverse biological disciplines such as molecular and cell biology, cytogenetics, biochemistry, development, evolution and biogeography, and it is also a very important and useful organism in Ecotoxicology and Aquaculture. *Artemia* is represented in South America by two bisexual species: *A. franciscana* (2n=42) and *A. persimilis* (2n=44). There are morphological, allozymic and cytogenetic differences between both species (ABREU-GROBOIS 1987; BADARACCO *et al.* 1987; BARATELLI and BARIGOZZI 1990; COLIHUEQUE and GAJARDO 1996a, 1996b; RODRÍGUEZ GIL *et al.* 1998; COHEN *et al.* 1999; PAPESCHI *et al.* 2000). *Artemia persimilis* is restricted to Argentina and a few Chilean localities (COHEN 1998; GAJARDO *et al.* 1998; COHEN *et al.* 1999; DE LOS RÍOS and ZUNIGA 2000; GAJARDO *et al.* 2001) while *Artemia franciscana* is widely distributed throughout the continent and has recently been cited for the first time for Argentina (PAPESCHI *et al.* 2000). The presence of *A. franciscana* was reported there in two Argentinean localities: Mar Chiquita and Las Tunas Lagoon (Córdoba province). The cytogenetic and morphological traits together with cross-breeding results and the biometrics of cysts and *nauplii* in the latter population led us to suggest an occasional hybridization between *A. franciscana* and *A. persimilis* (PAPESCHI *et al.* 2000).

One of the essential components of the genome is the heterochromatin, depending its biology not only on the repetitive DNA sequences that characterize it but also on its specific DNA binding proteins. Although many of its structural and functional characteristics are poorly known, its presence interferes with DNA replication, and contributes to chro-

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mosome structure, gene expression, genome organization and evolution (HENNIG 1999; REDI *et al.* 2001). Differences in heterochromatin content and distribution would be related to karyotype evolution and genetic differentiation among related species (REDI *et al.* 2001). In the genus *Artemia*, heterochromatin varies between and within species, and it has been suggested that this genomic trait would be correlated to some extent with genetic differentiation (GAJARDO *et al.* 2001).

In order to obtain more data on the geographical distribution of both species in our country, and to define the cytogenetic variability of both species and the potential existence of a hybrid zone, we analyzed four new populations of *Artemia* near Las Tunas. We analyzed the meiotic chromosomes and their behaviour in adult males from Salina La Antigua (La Rioja province), Salinas Grandes (Córdoba province), Pampa de las Salinas (San Luis province) and Salitral de la Vidriera (Buenos Aires province) and the mitotic chromosomes of *nauplii* from the last three populations. The heterochromatin content in the last three populations was analyzed by C and DAPI banding; furthermore, the results of silver staining on interphase nuclei of *nauplii* from them were also compared.

MATERIALS AND METHODS

Samples of cysts and adults of the brine shrimp were collected from Salinas Grandes (Córdoba province: 26°30'-31°40' S, 64°15'-66°25' W), Pampa de las Salinas (San Luis province: 32° S, 66°41' W) and Salitral de la Vidriera (Buenos Aires province: 38°42 S, 62°40 W) while only adults were collected from Salina La Antigua (La Rioja province: 29°54' S, 66°7' W) (Fig. 1).

Meiotic studies: adult males were fixed in 3:1 (absolute ethanol: glacial acetic acid) and kept at 4° C. Testes were dissected out, and meiotic slides were performed by placing a piece of gonad in a drop of iron propionic haematoxylin 2%. After slight dilaceration, the material was squashed under the coverslip. A total number of 7 adult males from La Antigua, 10 from Salinas Grandes, 20 from Pampa de las Salinas and 10 from Salitral de la Vidriera have been dissected, but only 5, 8, 18 and 3 specimens, respectively, showed meiotic cells suitable for chromosome analysis.

Mitotic studies: *nauplii* of 12-24 h from Salinas Grandes, Pampa de las Salinas and Salitral de la Vidriera were obtained from newly hatched cysts following standard procedures; they were treated with colchicine 0.05% following the technique of Colihueque and Gajardo (1996a, 1996b) and then they were squashed in a drop of iron propionic haematoxylin 2% for mitotic studies.

Fig. 1 — Location of Salina La Antigua (La Rioja province), Salinas Grandes (Córdoba province), Pampa de las Salinas (San Luis province) and Salitral de la Vidriera (Buenos Aires province) (Argentina).

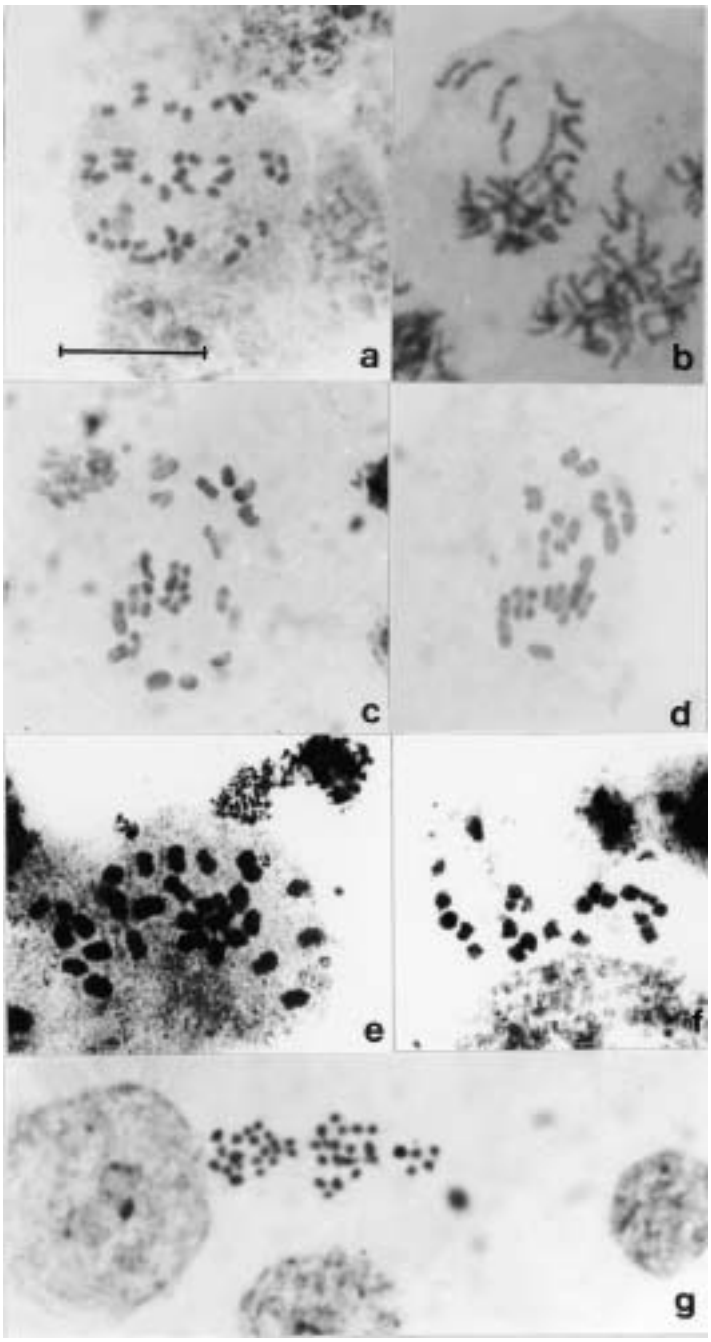

Fig. 2 — Mitotic and meiotic chromosomes of *Artemia* populations from Salina La Antigua (LA) (2n=42, n=21)(a-d), Pampa de las Salinas (PS) (2n=42, n=21) (e,f) and Salitral de la Vidriera (SV) (2n=44, n=22) (g). a) Spermatogonial prometaphase (LA). b) Pachytene showing complete pairing (LA). c) Diakinesis with 21 bivalents (LA). d) Prometaphase I with 21 bivalents (LA). e) Prometaphase I with 21 bivalents (PS). f) Prometaphase II with 21 chromosomes (PS). g) Mitotic prometaphase with 44 chromosomes (SV). Bar= 10 µm.

Some *nauplii* were squashed in 45% acetic acid and the coverslip was removed by the dry-ice method. These slides were then air-dried and kept for the banding techniques. C-banding, silver staining and fluorescent staining with the AT specific 4'6- diamidino-2-phenylindole (DAPI) were performed on *nauplii* interphase nuclei according to previous reports (Papeschi 1988, 1995; Papeschi *et al.* 2000).

RESULTS

The individuals from the populations of La Antigua, Pampas de las Salinas and Salinas Grandes have the chromosome number corresponding to *A. franciscana* (2n=42, n=21) (Fig. 2a-f). Diploid number in individuals from La Antigua was deter-

mined from spermatogonial prometaphases (Fig. 2a) since no cysts were available. The same diploid number was found in *nauplii* from Salinas Grandes and Pampa de las Salinas (not shown). During spermatogenesis chromosome pairing was complete (Fig. 2b) and meiosis was regular, with the formation of 21 bivalents (Fig. 2c-e). The regular meiotic behaviour was further confirmed when analyzing cells at prometaphase II, which always showed 21 chromosomes (Fig. 2f). On the other hand, the population of Salitral de la Vidriera presents the chromosome number characteristic of *A. persimilis* (2n=44, n=22) (Fig. 2g).

C-banding of interphase nuclei revealed the presence of numerous C positive chromocenters in Pampa de las Salinas (Fig. 3a-d) and Salinas Grandes (Fig. 3e-f) samples. No difference was detected between both populations with respect to the C heterochromatin content. Conversely, in Salitral de la Vidriera very scarce C positive heterochromatin was detected, and interphase nuclei showed from 0 to 2 small chromocenters (Fig. 3 g, h).

Similar results were obtained after DAPI staining; numerous DAPI bright chromocenters were observed in the interphase nuclei of *nauplii* from Salinas Grandes (Fig. 4 a-c) and Pampa de las Salinas (Fig. 4 d), while no DAPI bright signal was detected in Salitral de la Vidriera (Fig. 4e). Finally, silver staining revealed the presence of one up to four nucleoli in the three populations.

DISCUSSION

Seven bisexual species of the genus *Artemia* (Crustacea Branchiopoda, Anostraca) and many parthenogenetic forms with different ploidy levels, but collectively described as a unique binomen, *A. parthenogenetica*, are distributed in hypersaline environments in all continents except the Antarctic. For the New World two bisexual endemic species have been described, *Artemia franciscana* and *A. persimilis*. Until now, parthenogenesis in the genus is lacking in the American continent. Until recently it was assumed that *A. franciscana* was widely distributed in North, Central and South America except Argentina, whereas *A. persimilis* was restricted to this last country (VANHAECKE *et al.* 1987; COHEN *et al.* 1999).

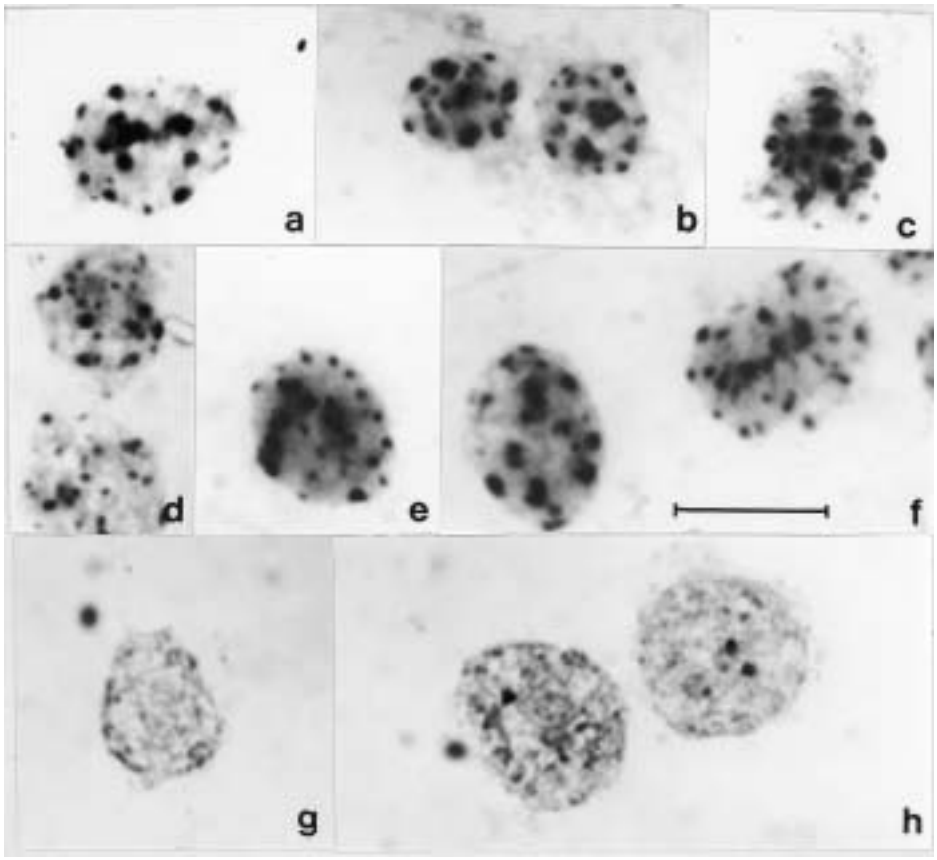

Fig. 3 — C banding of interphase nuclei of *nauplii* from Pampa de las Salinas (a-d), Salinas Grandes (e-f) and Salitral de la Vidriera (g-h). Bar= 10 µm.

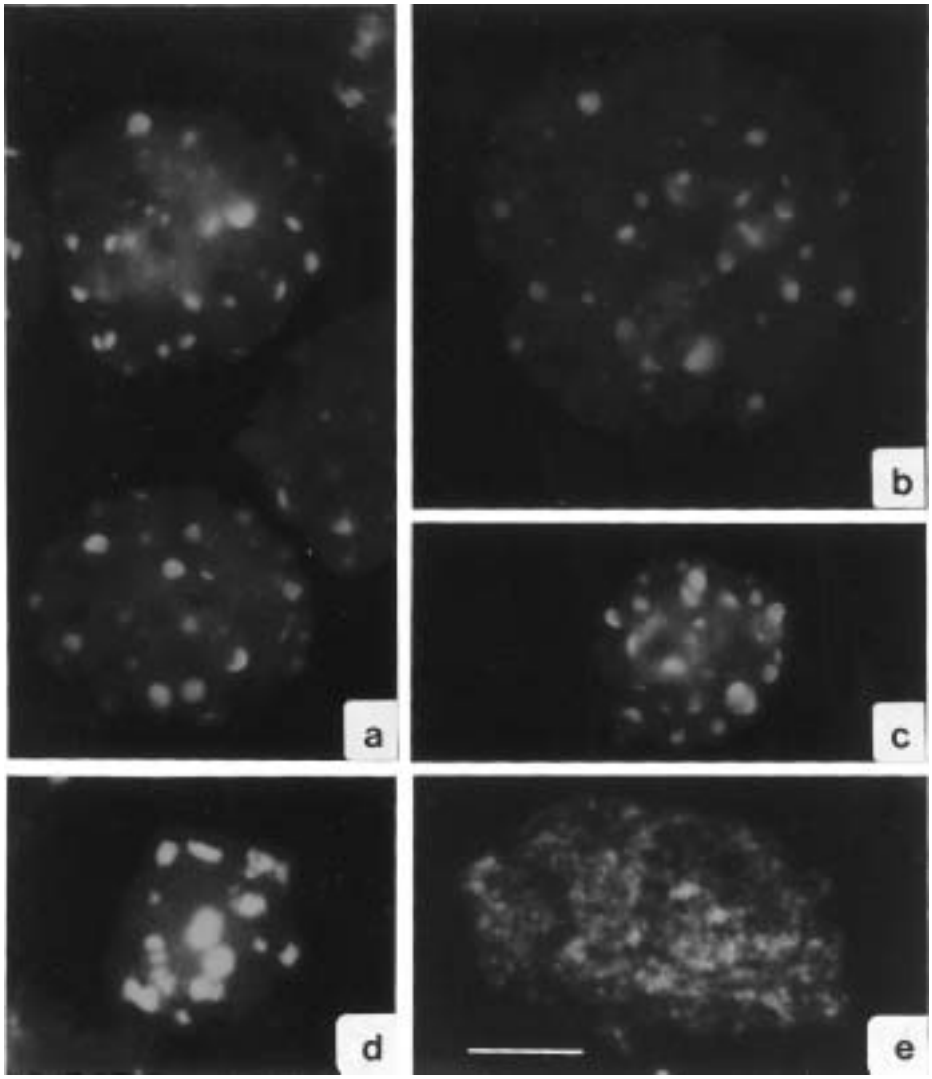

Fig. 4 — DAPI staining of interphase nuclei of *nauplii* from Salinas Grandes (a-c), Pampa de las Salinas (d) and Salitral de la Vidriera (e). Bar = 10 µm.

As new populations were recorded and analyzed this clear cut distribution pattern has been reconsidered. *A. persimilis* has now been reported in a few Chilean localities (Gajardo *et al.* 1998; De los Ríos and Zuniga 2000; Gajardo *et al.* 2001) while *A. franciscana* has also been cited in Argentina (Papeschi *et al.* 2000). Precisely, the presence of *A. franciscana* has been there recorded for the first time in our country from two locations in Córdoba province, Mar Chiquita and Las Tunas, the last probably representing a hybrid and/or introgressant population. Main objectives of our investigations are to determine the distribution of *Artemia* species in our country and the presence of a hybrid zone, as well as evaluate intra and interspecific genomic variability.

The cytogenetic analyses of four new populations of *Artemia* here reported reveal the presence of *A. franciscana* in three new locations: Salinas Grandes (Córdoba province), Pampa de las Salinas (San Luis province) and La Antigua (La Rioja province) and of *A. persimilis* in Salitral de la Vidriera (Buenos Aires province). Since male meiotic behaviour in all the populations was highly regular, no hybridization event seems to have occurred in any of them.

The silver staining technique, which reveals active NORs and nucleoli, was applied to interphase nuclei of *nauplii* from three populations (Salinas Grandes, Pampa de las Salinas and Salitral de la Vidriera). In all of them one and up to four nucleoli were detected. These observations suggest that both *A. franciscana* and *A. persimilis* have two pairs of chromosomes with active nucleolus organizer regions (NORs). Since nucleoli tend to associate their number varies from four nucleoli, when no associa-

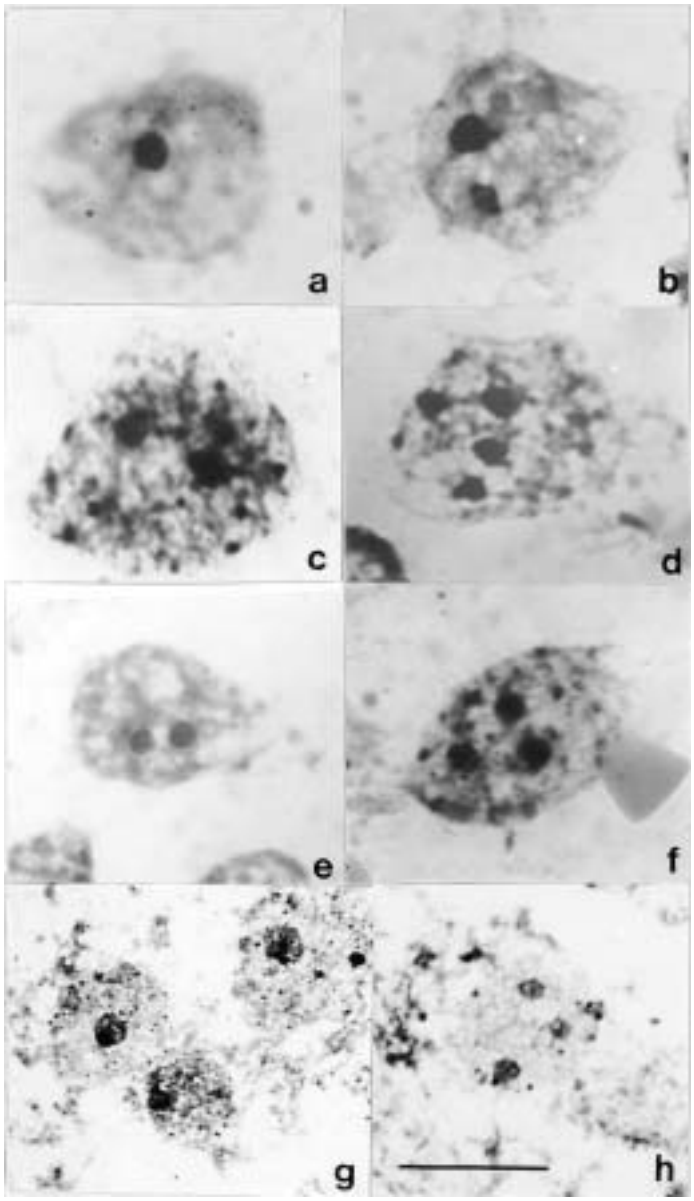

Fig. 5 — Cytogenetics of *Artemia* populations from Argentina. Lipko *et al.*

tion occurs, to only one nucleolus, when the four NOR chromosomes associate to form only one.

The number of chromocentres has been used to establish the taxonomical identity of *Artemia* populations. This number is positively correlated with the intensity of C-banding and with the amount of heterochromatin (Alu1 repetitive DNA fragment) present in different strains (Badaracco *et al.* 1987). The chromocentre number in *A. persimilis* populations ranges between 0 and 6, while in *A. franciscana* a polytypism for the number (between 4 and 17) and size of chromocentres has been described (Abreu-Grobois 1987; Colihueque and Gajardo 1996a, 1996b; Gajardo *et al.* 2001).

The three populations of *A. franciscana* here analyzed showed large amounts of C positive and DAPI bright heterochromatin, and no cytogenetic difference was detected among them. In agreement with our previous results in *A. franciscana* from Mar Chiquita and also in the peculiar population from Las Tunas, the Argentinean populations show a lower heterochromatin content than the individuals from the Great Salt Lake (USA) reference strain of *A. franciscana*. On the other hand, the population of *A. persimilis* from Salitral de la Vidriera showed one or two small chromocentres of small size. This observation differs from previous results of *A. persimilis* from Salinas Grandes, Hidalgo (La Pampa province)

in which C positive heterochromatin was completely absent (PAPESCHI *et al.* 2000).

Further studies are needed in order to clarify the relationship between heterochromatin content and geographical, ecological or environmental characteristics of the species under study.

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