*International Journal of Salt Lake Research* **7:** 41–44, 1998.


# *Artemia tibetiana*: preliminary characterization of a new *Artemia* species found in Tibet (People's Republic of China). International Study on *Artemia*. LIX *

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**Abstract.** In this study, we report on the existence of a new bisexual *Artemia* species, found in the high plateaus of Tibet (P. R. China). Different disciplines have been used to characterize this new population: biometrics of cysts and nauplii, morphometry of adults, cytogenetics, allozyme and DNA analyses and cross-breeding/fertility tests with known *Artemia* species. The results obtained justify that there is enough evidence to support the view that *Artemia* from Tibet is a new species with the proposed name *Artemia tibetiana*.

**Key words:** *Artemia*, brine shrimp, characterization, new species

The brine shrimp *Artemia* (Crustacea, Anostraca) inhabits both inland and coastal saline and hypersaline lakes. The genus is a complex of species and superspecies defined largely, though not completely, by the criterion of reproductive isolation (Browne and Bowen, 1991; Pilla, 1992).

*Artemia* bisexual species are grouped in the New World (NW) species, i.e. *Artemia franciscana* Kellogg (1906) and *A. persimilis* Piccinelli and Prosdocimi (1968), and in the Old World (OW) species, i.e. *A. salina* Leach (1819) (Triantaphyllidis et al., 1997b), *A. urmiana* (Günther, 1890), *A. sinica* (Cai, 1989) and *Artemia* sp. from Kazakhstan (Pilla and Beardmore, 1994), while all parthenogenetic forms are grouped, not very successfully, under the binomen *A. parthenogenetica*. All bisexual species are diploid (2n = 42) except for *A. persimilis* which has 2n = 44. In parthenogens, a great variety

* International interdisciplinary study on *Artemia* populations coordinated by the Laboratory of Aquaculture & Artemia Reference Center, University of Ghent, Belgium.

of ploidies has been observed (Barigozzi, 1974; Abatzopoulos et al., 1986; Triantaphyllidis et al., 1996), while in all *Artemia* species the phenomenon of aneuploidy, at least in the first larval stages (instar-I nauplii) is a quite common fact. The characterization of these species and/or populations has been a continuous task during the second half of this century and, recently, new multidisciplinary approaches were used to define *Artemia* species (for an extensive review see Triantaphyllidis et al., 1997b).

In this short paper, we report on the existence of a new bisexual *Artemia* species, found in Lagkor Co Lake on the high plateaus of Tibet (P.R. China). This new species has been characterized using different disciplines: i.e. biometrics of cysts and nauplii, morphometry of adults, cytogenetics, allozyme and DNA analyses and cross-breeding/fertility tests with known *Artemia* species. According to Mianping (1997), Lagkor Co is a carbonate lake, situated 4,490 m above sea level in the arid-temperate plateau zone of Tibet, at 84° 13′ E and 32° 03′ N. This salt lake has a salinity of (±) 60 g $L^{-1}$, with alkaline water (pH 8.8) and the temperature varies from a maximum of (±) 24 °C to a minimum of (±) – 26 °C, with average annual air temperature of (±) 1.6 °C (for more information on the lake's characteristics and hydrochemical type see Mianping, 1997). Dong et al. (1982) (cited in Mianping, 1997) characterized the *Artemia* population from Lagkor Co Lake as a cryophilic *A. salina*.

The cyst diameter of Tibet *Artemia*, determined on two samples collected in different time periods within the same year (1996), is the biggest ever recorded for both bisexual and parthenogenetic species: sample B: 323 $\mu$m (± 17.2), sample C: 330 $\mu$m (± 14.6). Also, the length of instar-I nauplii is the largest ever reported for *Artemia*: 667 $\mu$m (± 32.7). These unique characteristics seem to be species specific since they do not differ significantly in values recorded for cysts produced in laboratory culture, under different feeding regimes, or in nauplii hatched from these laboratory-produced cysts.

The total body length of both adult males and females seems to discriminate this species from the other known bisexual *Artemia* species. Preliminary results on several other morphologic characteristics with high diagnostic value, analyzed using multivariate and hierarchical cluster analyses (method in Triantaphyllidis et al., 1997a), support the above discrimination.

Chromosome preparations using the method described by Abatzopoulos et al. (1986) reveal that *Artemia* from Tibet is diploid having 42 chromosomes (2n = 42) with a high percentage of aneuploid nuclei; no chromocenters have been observed in resting nuclei, a fact rather expected for an *Artemia* species from Eastern Old World.

A total of 13 enzymes encoded by 20 loci were analyzed by gel electrophoresis (according to methods described by Abreu-Grobois and Beard-

more, 1980). The genetic distances (following the methodology described by Triantaphyllidis et al., 1997b) based on allozyme analyses show that *Artemia* from Tibet is well differentiated from the New World and Western Old World species (*A. franciscana* and *A. salina*) and seems to be closer to Eastern Old World species (*A. urmiana* and *A. sinica*).

Reproductive compatibility was evaluated from single-pair reciprocal crosses of adults between *Artemia* from Tibet (TIB) and 5 other bisexual species, i.e. *A. franciscana* (FRA), *A. salina* (SAL), *A. urmiana* (URM), *A. sinica* (SIN) and *Artemia* sp. from Yimeng (YIM). The crosses performed were: intraspecific: FRA/FRA, SAL/SAL, URM/URM, SIN/SIN, YIM/YIM and TIB/TIB as controls; interspecific: TIB/FRA, FRA/TIB, TIB/SAL, SAL/TIB, TIB/URM, URM/TIB, TIB/SIN, SIN/TIB, TIB/YIM and YIM/TIB (the first three letters represent the male). Crosses were inferred to be fertile when live nauplii or full cysts were produced. The results reveal that: (1) complete infertility exists between Tibet *Artemia* and *A. salina*; the same is valid between Tibet *Artemia* and *A. franciscana* with one exception (a female *A. tibetiana* and a male *A. franciscana* generated 13 sterile F1 nauplii which died within 72 hrs); these data must be the result of substantial post-mating reproductive isolation under laboratory conditions since pairing was always observed; and (2) when matings between *Artemia* from Tibet and the other Eastern Old World species were considered, the proportion of interspecific crosses which was fertile (40–60 per cent) was found to be lower than that of the intraspecific controls (but not significantly lower in all cases). This indicates that post-mating isolating barriers to gene flow are not so strong, at least in the laboratory level and especially during F1 since in several cases there was apparent hybrid breakdown at F2 and F3 generations.

Based upon the data presented above, which are, also, reinforced by the results from DNA analysis (study of the genomic DNA polymorphisms of *Artemia* species using RAPD markers), we consider that there is enough evidence to support the view that *Artemia* from Tibet is a new species with the proposed name *Artemia tibetiana*.

## Acknowledgements

We would like to express our thanks to Prof. J.A. Beardmore, Dr. P. Bossier, Dr. G.V. Triantaphyllidis and Mr. I. Kappas for their substantial help through useful discussions and suggestions. The technical help of Mr. K. Koudouris and the sample collection by Prof. Zheng Mianping are also acknowledged. This study was supported by the Flemish Science Council (project G.0260.97), the Bilateral Scientific and Technological Cooperation Flanders – China and the State Science and Technology Commission of China.

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