A new gynandromorphic morphological pattern in *Artemia* Leach, 1819 (Crustacea: Anostraca)

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An *Artemia* specimen displaying a new type of gynandromorphic morphology was found in a parthenogenetic *Artemia* cultured population from Ga Hai, Qinghai, China. The specimen possesses two male second antennae, but the genital segments are bilaterally gynandromorphic, with the right side bearing a half ovisac and the left side a single gonopod (penis).

**Key words:** *Artemia*, gynandromorphy, morphology, SEM.

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**INTRODUCTION**

Gynandromorphs, also known as sexual mosaics (Bowen & Hanson, 1962; Wieschaus & Gehring, 1976; Sassa -man & Fugate, 1997; Maeno & Tanaka, 2007; Narita et al., 2010), are genetically chimeric individuals consisting of distinct male and female tissues whose phenotype is determined by their own genotypes (Narita et al., 2010). They differ from intersexes by that an intersex case is genetically uniform (complete male, complete female or intermediate in every tissue) but all/some parts of their tissues have either a sexual phenotype opposite to their genetic sex or an intermediate sexual phenotype (Narita et al., 2010; see also Krumm, 2013). In cases that the genetics of the sexual heterogeneity is unknown, the gynandromorph (morphologic/phenotypic) is usually defined as that the morphological borders between the female and male parts is clear, while an intersex is a phenotype intermediate to normal male and female characteristics (Narita et al., 2010, see also Krumm, 2013). In general, gynandromorphs originate from the damage or loss of a sex chromosome during cleavage or from binucleate eggs (Blanchard & Descimon, 1988; Bridgehouse, 2000; Narita et al., 2010), but gynandromorphism is also known to be induced by *Wolbachia*-infection in some insects (see Yoshizawa et al., 2009; Narita et al., 2010; Bear & Monteiro, 2013), although this is not likely in *Artemia* (Maniatsi et al., 2010). The discovery of a gynandromorphic specimen is an out of ordinary event in nature and even in laboratory rearing (Bridgehouse, 2000; Narita et al., 2007; Ali & Rasheed, 2008).

Individuals representing five gynandromorphological types have been recorded thus far in *Artemia* species (Bowen & Hanson, 1962; Bowen et al., 1966; Liu et al., 2005; Campos-Ramos et al., 2006). Herein we present a new gynandromorphic pattern in *Artemia*.

**MATERIALS AND METHODS**

Parthenogenetic *Artemia* from Ga Hai (Delingha, Qinghai, China; 37°08’N, 97°33’E) was cultured under laboratory conditions (salinity: 70 ppt; temperature: 25°C) for various studies. A gynandromorphic specimen was found in one of the cultures. The morphological structure of head (secondary sexual characteris-
tic) was photographed using a Nikon SMZ-800 stereomicroscope equipped with a camera. Three normal females and three normal rare males were examined for comparison with the gynandromorphic specimen. Specimens for Scanning Electron Microscope (SEM) observation were fixed with 4% formaldehyde. After washing with phosphate buffer and re-distilled water, they were dehydrated in 80%, 90% and 100% ethanol at 30 min intervals. Then samples were transferred into isooamyl acetate:ethanol (1:3, 1:2 and 1:0 for 30 min, respectively). After dried with a Hitachi HCP-2 critical point dryer, specimens were observed and photographed with a KYKY-2800B Scanning Electron Microscope (SEM).

RESULTS

Our gynandromorph specimen possesses two male second antennae (Fig. 1), has the same head morphology as that in typical male *Artemia*. The genital segments exhibit a bilateral pattern of gynandromorphy in external reproductive organs. On the right side, an incomplete brood pouch is present with a single spine pointing toward the sagittal plane. On the left side a single gonopod (penis; for extensive reviews see Rogers et al., 2007) is present (Fig. 2). The gynandromorph’s gonopod is not appreciably different from that of a typical male (see Figs 2 and 3). Ovisac spines in gynandromorphic and normal specimens have similar anatomical complex: the proximal part is characterized with a well expanded pedestal and the distal part is characterized with a smooth surface (Fig. 4A and B). Similarly there is not any obvious difference between the apical part of gonopod spines (Figs 2A and 3). No oocyte was observed in the imperfect ovisac. The ovisac length is 0.76 mm and the gonopod length is 0.29 mm, whereas in normal females and rare males are 1.6 ± 0.2 mm (n = 3) and 0.47 ± 0.05 mm (n = 4), respectively. The length of gynandromorph's ovisac spine is 94.8 µm [169.2 ± 22.7 µm (n = 3) in normal females].

DISCUSSION

The earliest example of gynandromorphy is a 70 million year old fossil of a crab (Bishop, 1973; see also Ford, 2012). Although most records of gynandromorphs are arthropods (Scriber & Evans, 1988; Bridgehouse, 2000; Narita et al., 2010; Giangarelli & Sofia, 2011; Yuan et al., 2011), there are some examples in other animals like lizards and birds (DaCosta et al., 2007; Argaña et al., 2013). Gynandromorphs are classified...
into two main groups according to the distribution of female and male primary/secondary sexual characteristics: bilateral (left/right) and non-bilateral. The latter category can be further divided into axial (anterior/posterior) and mosaic (patchy pattern) gynandromorphy (see Belk, 1978; Sassaman & Fugate, 1997; Krumm, 2013).

The first gynandromorphic *Artemia* specimen was found in an X-ray irradiated culture of *Artemia franciscana* by Bowen & Hanson (1962). It was a bilateral gynandromorph, with the right side of body bearing a normal male and the left side a normal female morphology. Bowen *et al.* (1966) studied the morphological abnormality of wild and X-irradiated bisexual *Artemia* (*Artemia monica*, *Artemia franciscana*, *Artemia persimilis* and *Artemia salina*). Although 11 sex mosaics have been documented in their study, only four samples could be defined rigorously as morphological gynandromorphs and the others might be intersexual individuals. Three of the four gynandromorphs were bilateral (Bowen *et al.*, 1966: Table III, no. 8, 9, 10) and the other one had paired gonopods and bilateral second antennae (Bowen *et al.*, 1966: Table IV, no. 18). Liu *et al.* (2005) described two gynandromorphs from the parthenogenetic *Artemia* population of Ga Hai. Both had paired male antennae and two gonopods, but the left side of the abdomen bore an ovary containing several oocytes. The last three gynandromorphs were described by Campos-Ramos *et al.* (2006) from both parthenogenetic *Artemia* (two specimens) and *Artemia franciscana* (one specimen). One of the specimens from parthenogenetic *Artemia* had normal ovisac and gynandromorphic antennae, while the other had normal female head and gynandromorphic genital segments (imperfect brood pouch on the right and a gonopod-like structure on the left). The gynandromorph specimen of *A. franciscana* was morphologically female in the genital segments and bilateral in the antennae. Table 1 is a summary of gynandromorphs recorded in the genus *Artemia*. The present specimen, which has the head pattern of a normal male and the genital segments bearing a half ovisac on the right and a single gonopod on the left, is a new mosaic pattern of gynandromorphism in *Artemia*.

In addition to *Artemia*, gynandromorphs have been reported also in other Anostraca species of Chirocephalidae and Branchinectidae. Only bilateral gynandromorph was presented for the chirocephalid species (*Chirocephalus diaphanus*, *Eubranchipus serratus* and *E. vernalis*) (Gissler, 1881; Dexter, 1953; Nouris-
A. Asem and S.C. Sun — A new gynandromorphic morphological pattern in Artemia

son & Lenel, 1968; Cottarelli & Mura, 1972; Thiéry, 1985; Sassaman & Fugate, 1997). In Branchinectidae axial gynandromorphs (two specimens) and mosaic gynandromorphs (one specimen) have been recorded for Branchinecta (Sassaman & Fugate, 1997; Krumm, 2013). Although axial gynandromorphs had been imputed to the existence of an epigenetic feminizing factor that acted on thoratic segments after the cellular determination of cephalic segmentation (Sassaman & Fugate, 1997), Krumm (2013) demonstrated that the sex determination of Branchinecta was more likely cell autonomous. Bilateral gynandromorphy had been referred to the loss or damage of a sex chromosome during first zygotic division (Bowen & Hanson, 1962; Scriber & Evans, 1988; Bridgehouse, 2000; Narita et al., 2010). The brine shrimp Artemia has a WZ-ZZ sex-determining system (Saavedra & Amat, 2005; De Vos et al., 2013). In parthenogenetic Artemia (with a genotype of WZ), the formation of bilateral gynandromorphs may result from the loss or damage of the W chromosome during first division of eggs. Similarly mosaic gynandromorphy, as that described in the present note, is probably due to the loss/damage of this chromosome in later divisions of cleavage.

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