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— SHORT COMMUNICATION —

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.

INTRODUCTION

Gynandromorphs, also known as sexual mosaics (Bowen & Hanson, 1962; Wieschaus & Gehring, 1976; Sassaman & 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-

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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 isoamyl 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).

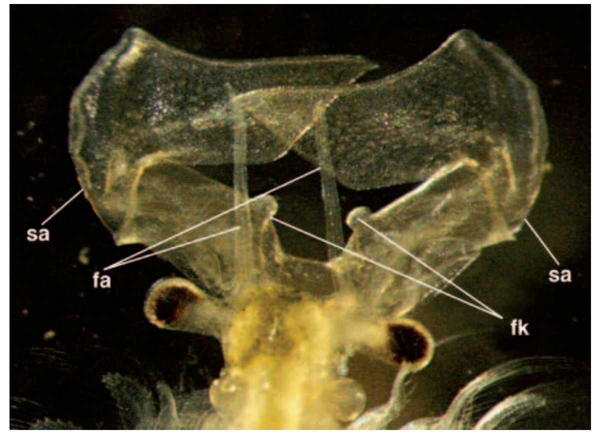

FIG. 1. Dorsal view of head in the gynandromorphic specimen of *Artemia* from Ga Hai. sa: second antenna, fa: first antenna, fk: frontal knob.

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

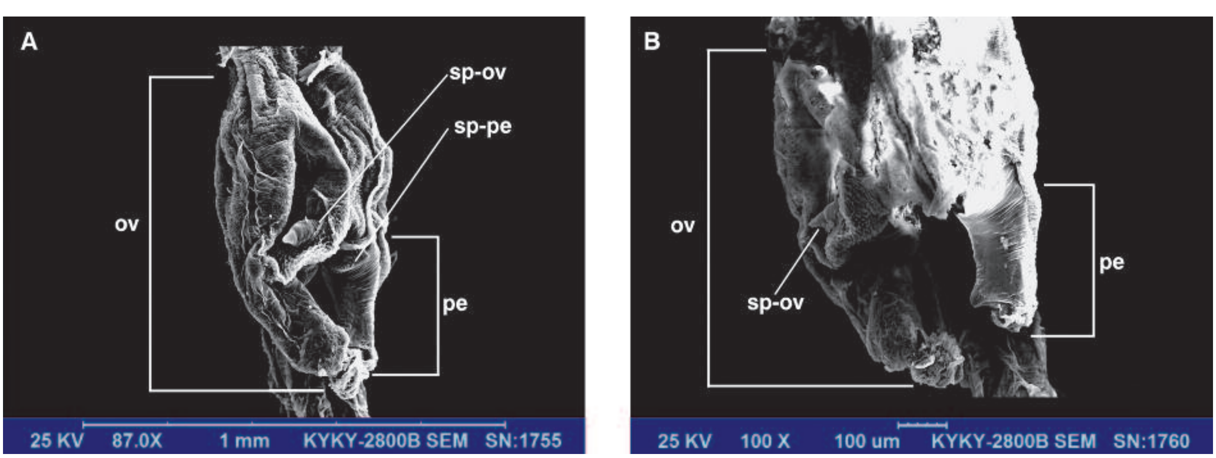

FIG. 2. SEM photographs of external reproductive organs of the gynandromorphic *Artemia* specimen from Ga Hai. A) ventral view; B) ventrolateral view. ov: ovisac, sp-ov: spine of ovisac, pe: gonopod, sp-pe: spine of gonopod.

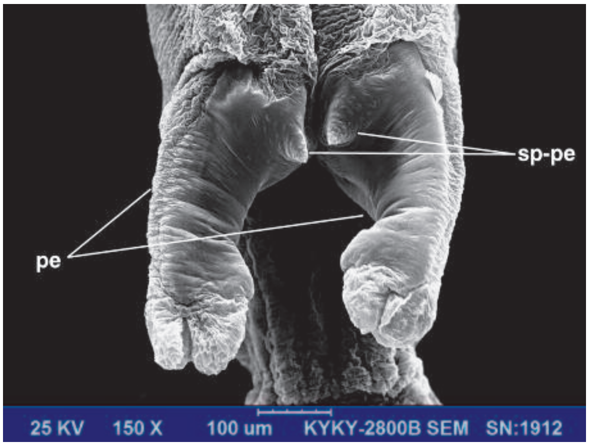

FIG. 3. SEM photograph of gonopods in normal rare male of parthenogenetic *Artemia* from Ga Hai. pe: gonopod, sp-pe: spine of gonopod.

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 gynandromorphy was presented for the chirocephalid species (*Chirocephalus diaphanus*, *Eubranchipus serratus* and *E. vernalis*) (Gissler, 1881; Dexter, 1953; Nouris-

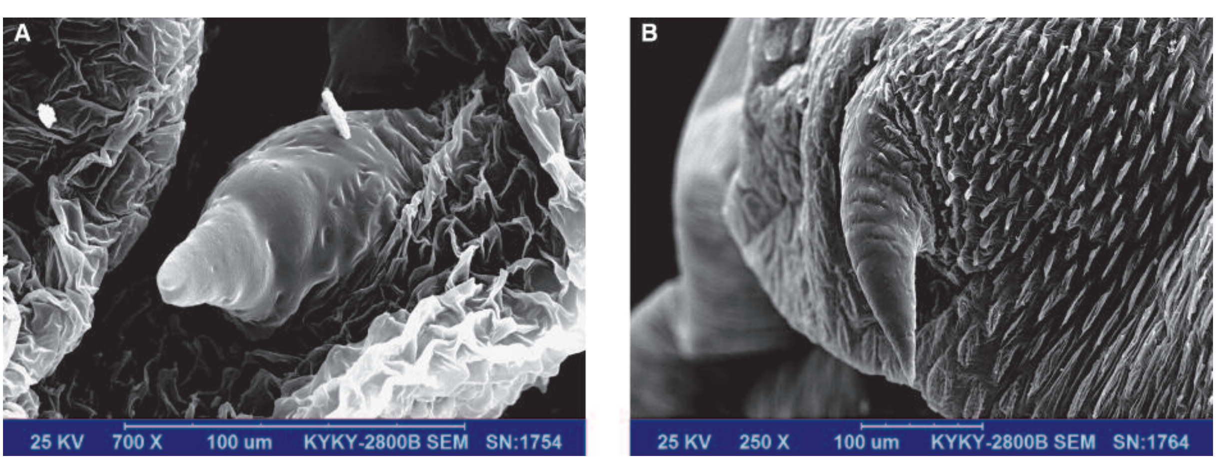

FIG. 4. SEM photographs of ovisac spine of the parthenogenetic *Artemia* from Ga Hai. A) gynandromorphic specimen; B) normal female.

TABLE 1. Reported patterns of gynandromorphism in brine shrimp *Artemia*

Taxon	Locality	Individuals	General pattern	Structure of head	Structure of genital segments	References
A. franciscana	California†, USA	1	bilateral	bilateral	bilateral	Bowen & Hanson (1962)
A. franciscana	hybrid population§	1	non-bilateral (mosaic)	bilateral	male	Bowen *et al*. (1966)
Artemia sp.ʃ	Unknown	3	bilateral	bilateral	bilateral	Bowen *et al*. (1966)
Parthenogenetic	Ga Hai, China	2	non-bilateral (mosaic)	male	two gonopods and one ovary	Liu *et al*. (2005)
Parthenogenetic	Great Salt Lake*, USA	1	non-bilateral (mosaic)	bilateral	female	Campos-Ramos *et al*. (2006)
Parthenogenetic	Great Salt Lake*, USA	1	non-bilateral (mosaic)	female	bilateral	Campos-Ramos *et al*. (2006)
A. franciscana	Great Salt Lake*, USA	1	non-bilateral (mosaic)	bilateral	female	Campos-Ramos *et al*. (2006)
Parthenogenetic	Ga Hai, China	1	non-bilateral (mosaic)	male	bilateral	present study

† Unknown locality

§ The original stock was a hybrid between San Franciscao Bay and Great Salt Lake populations [for more information, see Brown (1964)].

ʃ In this study *A. franciscana*, *A. persimilis*, *A. monica* and *A. salina* from several sites had been used [for more information see Brown (1964, 1965)].

* Commercial cysts from the Great Salt Lake

son & Lenel, 1968; Cottarelli & Mura, 1972; Thiéry, 1985; Sassaman & Fugate, 1997). In Branchinectidae axial gynandromorphs (47 specimens), bilateral 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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REFERENCES

Ali N, Rasheed SB, 2008. A gynandromorph of *Culex pipiens quinquifasciatus* from Pakistan. *Pakistan Entomologist*, 30: 1-4.

Argaña E, Freitas S, Sillero N, Corti C, Drovetski SV, Garcia-Muñoz E, Ferreira D, Ferreira S, Carretero MA, 2013. An apparent case of bilateral gynandromorphy in the femoral pores of the Caucasian rock lizard *Darevskia raddei*. *Herpetology Notes*, 6: 77-80.

Bear A, Monteiro A, 2013. Both cell-autonomous mechanisms and hormones contribute to sexual development in vertebrates and insects. *BioEssays*, 35: 725-732.

Belk D, 1978. A gynandromorph of the fairy shrimp *Branchinecta lindahli* Packard, 1883 (Anostraca). *Crustacea-*

na, 35: 319-321.

Bishop GA, 1973. A sexually aberrant crab (*Dakoticancer overanus* Rathbun, 1917) from the upper cretaceous Pierre Shale of South Dakota. *Crustaceana*, 26: 212-218.

Blanchard R, Descimon H, 1988. Hybridization between two species of swallowtails, meiosis mechanism, and the genesis of gynandromorphs. *Journal of the Lepidopterists' Society*, 42: 94-102.

Bowen ST, 1964. The genetics of *Artemia salina*. IV. Hybridization of wild populations with mutant stocks. *Biological Bulletin*, 126: 333-344.

Bowen ST, 1965. The genetics of *Artemia salina*. V. Crossing over between the X and Y chromosomes. *Genetics*, 52: 695-710.

Bowen ST, Hanson J, 1962. A gynandromorph of the brine shrimp, *Artemia salina*. *Genetics*, 47: 277-280.

Bowen ST, Hanson J, Dowling P, Poon M-C, 1966. The genetics of *Artemia salina*. VI. Summary of mutations. *Biological Bulletin*, 131: 230-250.

Bridgehouse DW, 2000. Bilateral gynandromorph of *Hyalophora cecropia* (Lepidoptera: Saturniidae) in Nova Scotia. *Northeastern Naturalist*, 7: 237-240.

Campos-Ramos R, Maeda-Martínez AM, Obregón-Barboza H, Murugan G, Guerrero-Tortolero DA, 2006. First report of gynandromorphy in parthenogenetic *Artemia* (Branchiopoda: Anostraca). *Journal of Crustacean Biology*, 26: 107-112.

Cottarelli V, Mura G, 1972. Un caso di ginandromorfismo in *Chirocephalus diaphanus* Prevost. *Rivista di Idrobiologia*, 65: 225-234.

DaCosta JM, Spellman GM, Klicka J, 2007. Bilateral gynandromorphy in a white-ruffed manakin (*Corapipo altera*). *The Wilson Journal of Ornithology*, 119: 289-291.

Dexter RW, 1953. Studies on North American fairy shrimps with the description of two new species. *American Midland Naturalist*, 49: 751-769.

De Vos S, Bossier P, Van Stappen G, Vercauteren I, Sorgeloos P, Vuylsteke M, 2013. A first AFLP-based genetic linkage map for brine shrimp *Artemia franciscana* and its application in mapping the sex locus. *PLoS ONE*, 8: e57585.

Ford AT, 2012. Intersexuality in Crustacea: An environmental issue? *Aquatic Toxicology*, 108: 125-129.

Giangarelli DC, Sofia SH, 2011. First record of a gynandromorph orchid bee, *Euglossa iopoecila* (Hymenoptera: Apidae: Euglossini). *Annals of the Entomological Society of America*, 104: 229-232.

Gissler CF, 1881. Description of a hermaphroditic phyllopod crustacean (*Eubranchipus*). *American Naturalist*, 15: 136-139.

Krumm JL, 2013. Axial gynandromorphy and sex determination in *Branchinecta lindahli* (Branchiopoda: Anostraca). *Journal of Crustacean Biology*, 33: 303-308.

Liu F, Xiang J, Bu W, Ji B, Liu A, 2005. Gynandromorphs of the brine shrimp *Artemia parthenogenetica* in Gahai Lake, Qingdao. *Marine Sciences-Qingdao*, 29: 73-75.

Maeno K, Tanaka S, 2007. Morphological and behavioural characteristics of a gynandromorph of the desert locust, *Schistocerca gregaria*. *Physiological Entomology*, 32: 294-299.

Maniatsi S, Bourtzis K, Abatzopoulos TJ, 2010. May parthenogenesis in *Artemia* be attributed to *Wolbachia*? *Hydrobiologia*, 651: 317-322.

Narita S, Nomura M, Kageyama D, 2007. A gynandromorph of *Eurema hecabe* (Lepidoptera: Pieridae) from Japan. *Entomological News*, 118: 134-138.

Narita S, Pereira RAS, Kjellberg F, Kageyama D, 2010. Gynandromorphs and intersexes: potential to understand the mechanism of sex determination in arthropods. *Terrestrial Arthropod Reviews*, 3: 63-96.

Nourisson M, Lenel R, 1968. Sur un cas de gynandromorphisme naturel chez *Chirocephalus* diaphanus Prev. (Crustacé, Branchiopode). *Annales de Limnologie*, 4: 105-112.

Rogers CD, Timms BV, Jocqué M, Brendonck L, 2007. A new genus and species of branchipodid fairy shrimp (Crustacea: Branchiopoda: Anostraca) from Australia. *Zootaxa*, 1551: 49-59.

Sassaman C, Fugate M, 1997. Gynandromorphism in Anostraca: multiple mechanisms of origin? *Hydrobiologia*, 359: 163-169.

Saavedra C, Amat F, 2005. Maternal effects on encystment in crosses between two geographic strains of *Artemia franciscana*. *Journal of Heredity*, 96: 713-717.

Scriber MJ, Evans MH, 1988. Bilateral gynandromorphs, sexual and/or color mosaics in the tiger swallowtail butterfly, *Papilio glaucus* (Lepidoptera: Papilionidae). *Journal of Research on the Lepidoptera*, 26: 39-57.

Thiéry A, 1985. Un cas de gynandromorphisme naturel chez *Chirocephalus diaphanus* Prevost (Crustacea, Anostraca). *Rivista di Idrobiologia*, 22: 83-87.

Wieschaus E, Gehring W, 1976. Gynandromorph analysis of the thoracic disc primordia in *Drosophila melanogaster*. *Wilhelm Roux's Archives*, 180: 31-46.

Yoshizawa J, Mimori K, Yamauchi K, Tsuchida K, 2009. Sex mosaics in a male dimorphic ant *Cardiocondyla kagutsuchi*. *Naturwissenschaften*, 96: 49-55.

Yuan F, Wang C, Zhang Y, Zhu C, 2011. A gynandromorphy of *Teleogryllus emma* (Ohmachi & Matsumura, 1951) (Orthoptera: Gryllidae) in China. *Entomotaxonomia*, 33: 101-102.