

Facultative sex reversal in the freshwater plankton *Sinodiaptomus* (*Rhinediaptomus*) *indicus* Calanoida: Copepoda

Gonochorism and sexual dimorphism are well pronounced in freshwater copepods, principally by their variation in size, modified antennule, asymmetrical fifth pleopod, number of urosomite, metasomal wings, caudal furca, genital complex and tegumentary gland¹⁻³. Since copepods face a wide range of fluctuation in environmental conditions in the habitat, they show a wide array of physiological adaptations, like the ability to reduce metabolic rate and enter into diapause³ tolerance to anoxia⁴, and behaviour alteration⁵. However, numerous morphological anomalies like congenital malformation, gynandromorphisms, intersexuality⁶, parasitic castration⁷, regeneration after injury² and sex change² have been reported.

Sinodiaptomus (*Rhinediaptomus*) *indicus* is one of the dominant and most common diaptomid inhabiting freshwater bodies of South India. In the population of *S. (R.) indicus* moderately to highly skewed female sex ratio is not uncommon^{1,8}. Highly skewed female sex ratio seasonally has prompted speculation about sex change in free-living copepod^{9,10}. According to Fleminger⁹, the available evidence for sex change in copepod is not conclusive. Moreover nothing is definitely known about the mechanisms of sex determination in copepods. The present work demonstrates the direct evidence for facultative sex change in the freshwater diaptomid by reporting functional female with male dimorphic characters.

Population dynamics and sex ratio of *S. (R.) indicus* were studied for a period of one year from May 1995 to May 1996 in a fish pond at Chetput Hydrobiological Station, Tamil Nadu State Fisheries located at the heart of Chennai. Zooplankton were collected in early hours of the day by filtering 200 l of pond water using 100-micron mesh plankton net. The samples were preserved in 5% buffered formalin. In the laboratory, samples were scanned under stereozoom dissection microscope. *S. (R.) indicus* were identified following the standard taxonomic keys^{2,11}. The sexes were identified based on the size, number of urosomal segments, symmetrical and asymmetrical antennule and fifth pleopod. Further morphological details like total length

and width were recorded using a calibrated ocular micrometer. The total length was taken from the tip of the forehead to the end of the caudal plate. The prosome width is measured at the broadest point of the prosome.

During the study period, *S. (R.) indicus* with both male and female features were observed in samples collected from Chetput fish pond. Further, similar types with both male and female characters were also observed in the laboratory-cultured animals. To ascertain the sex, the animals were counter-stained with haematoxylin

and eosin following the procedure of Peter Gray¹², for studying the gonads. For scanning electron microscope studies the animals were fixed in 2.5% glutaraldehyde at 4°C for 48 h and washed in 1 M phosphate buffer solution, osmicated in 1% osmium tetra-oxide, and dehydrated in ascending series of ethyl alcohol. The specimen was further treated with propylene oxide and air-dried below room temperature. The dried specimens were coated with palladium gold to thickness of 200 Å in ion sputtering device (JEOL JFC). The specimens were scan-

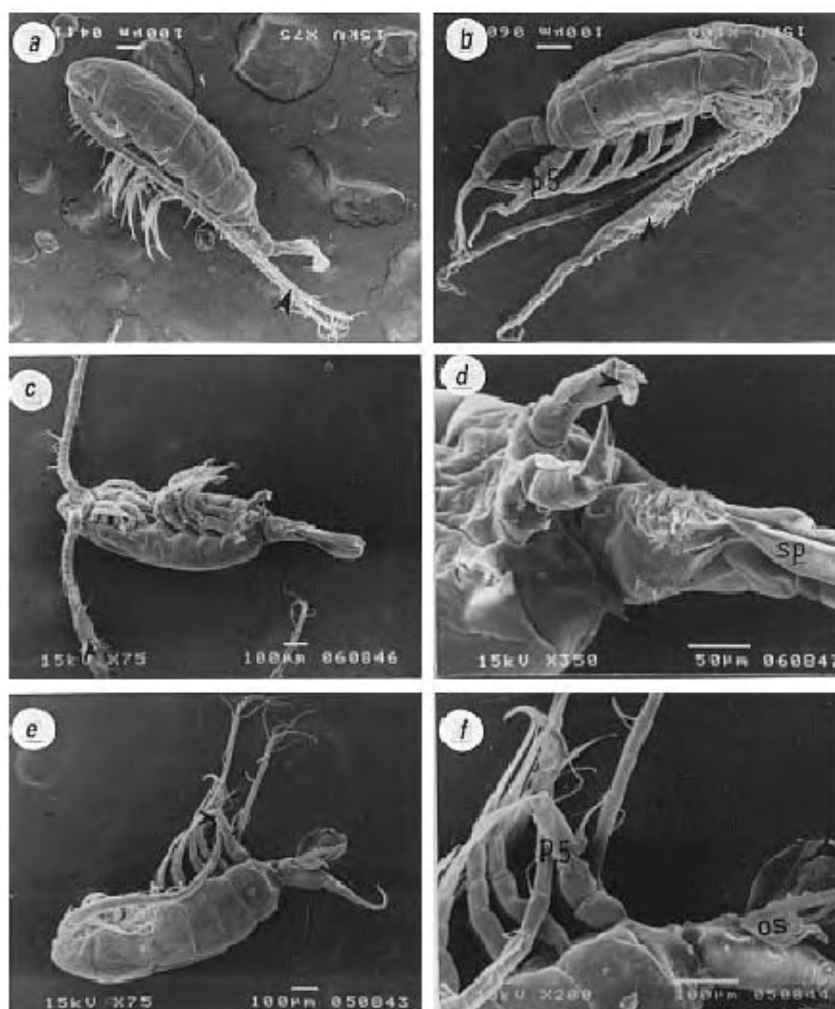


Figure 1. *a*, *Sinodiaptomus* (*Rhinediaptomus*) *indicus* adult female. Note normal antennule (arrow); *b*, Adult male. Note modified right antennule (arrow) and modified fifth pleopod (p5); *c*, Transformed female. Note modified male geniculated antennule (arrow); *d*, Transformed female. Note malformed left leg (arrow) and spermatophore attached to genital pore (sp); *e*, Transformed female. Note modified male fifth pleopod (arrow); *f*, Transformed female. Note higher magnification of modified male fifth pleopod (p5) and hatched-out ovisac attached to genital pore (os).

ned under scanning electron microscope (JEOL JSM 5300) at 15 kV.

The population of *S. (R.) indicus* in the Chetput fish pond during the study period ranged from 5 to 67 individuals per litre. The maximum density of 64 and 67 animals was recorded in May and September 1995, respectively. Throughout the study period the females outnumbered the males and occurred in the ratio of 3 : 1, except during September 1995. Females with morphological anomalies were observed during September 1995 at a rate of 5 ± 2 individuals per 100 number of individuals.

Three different kinds of morphological anomalies were recorded in the animals collected from the wild as well as those reared in the laboratory. All the animals with morphological anomalies were found to be females with regard to the number of urosomal segments and often they possessed ovisac with 20 ± 5 developing eggs. Light microscopic examination of specimen with morphological anomalies after counterstaining with haematoxylin and eosin revealed the presence of well-developed functional reproductive system with developing oocytes in the ovary and oviduct.

The genotypic female measures around $1400 \pm 100 \mu\text{m}$ in length and $360 \pm 50 \mu\text{m}$ in width (Figure 1 c) and is normally larger than the male. The urosome is 3-segmented, with a broad genital segment. Antennule is symmetrical and has 25 segments. The fifth pleopod is small in size and differs structurally from other swimming legs. It is comprised of coxopodite, basipodite, exopodite and endopodite. The endopodite is reduced, whereas the exopodite has two segments, with the terminal segment modified into the claw. The outer and inner margins of the claw are spinulated.

The genotypic male is comparatively ($1200 \pm 100 \mu\text{m}$ in length and $300 \pm 50 \mu\text{m}$ in width, Figure 1 b) smaller than the female, the urosome has 4 segments. Antennule is asymmetrical; the left antennule is similar to that in the female, whereas the right antennule is highly modified and geniculated. The number of segments is reduced to twenty-two due to fusion of 19th with 21st segment and 22nd with 23rd segment, resulting in two compound segments. Segments 13 and 18 are enlarged and a movable articulation occurs between the 18th and 19th segment. The distal end of the 23rd segment bears a comb-like hyaline process with nine linear teeth. The fifth leg is

asymmetrical and highly modified. The right leg is much larger than the left. The terminal segment of the left endopodite is modified into a strong S-shaped claw. The terminal segment of the right endopodite is modified into a thumb like process with lamellar surface.

Three different kinds of anomalies were recorded: the total length and pro-some width of these females measure $1300 \pm 100 \mu\text{m}$ and $300 \pm 50 \mu\text{m}$, respectively. The first type of female possessed all morphological characters of a normal female; however, the right antennule is geniculated as found in the normal adult male (Figure 1 c). Malformation is also observed in the distal part of the left fifth pleopod (Figure 1 d). The second type of female possessed normal symmetrical antennule but has modified asymmetrical fifth pleopod similar to that of males (Figure 1 e, f). The third type of female possessed both modified right antennule and fifth pleopod similar to that of normal adult males.

In general the sex of individuals may be determined genetically, often by the chromosomal mechanisms or epigenetically under the influence of environmental factors. In the present study there is skewed female sex ratio throughout the year (male : female ratio 1 : 3); similar sex ratio is also reported in another diaptomid *Heliodiaptomus vidus*⁸. The higher female sex ratio may be a function of the species to build up the population rapidly during favourable environmental conditions. The lower male ratio in *S. (R.) indicus* may be due to multiple mating efficiency and higher reproductive potential¹ of this species compared to other copepods⁸. In the present study *S. (R.) indicus* shows transformation from male to female, which obviously indicates that all the three types of females may be genetic male, but transform into functional female. Post-embryonic development of this species shows that male completes development within a short duration compared to females, and attains maturity first¹. A fraction of males in the absence of females might facultatively undergo sex change to become functional females. However, part of male secondary sexual characters like geniculated antennule and modified fifth pleopod is retained. Fleminger⁹ has suggested a hypothetical selective process in family Calanidae, where a fraction of genetic males changes to functional females. Males that switch over to functional

females retain part of the male characters and mate with normal males to produce offspring. The observations in the present study add supportive evidence to this hypothesis, as all three types of transformed females showed normal female reproductive system and ovisac containing developing embryos, even though they retain male geniculate appendages. Although parasitic castration by cystode cystecercoid stage in freshwater calanoid copepods is reported⁷, such a process is not indicated in *S. (R.) indicus* as they were free from any parasitism. Further studies are necessary to ascertain the stage at which sex reversal occurs as well as to understand the factors which induce the transformation and retention of male characters.

1. Dharani, G., Ph D, thesis, University of Madras, 1998, p. 141.
2. Dussart, B. H. and Defay, D., *Copepoda: Introduction to Copepoda*, SPB Academic Publication, The Netherlands, 1995.
3. Williamson, C. E., *Copepoda: Ecology and Classification of North American Freshwater Invertebrates*, Academic Press, San Diego, 1991, pp. 787–822.
4. Hicks, G. R. F. and Coull, B. C., *Mar. Biol.*, 1982, **21**, 67–175.
5. Wong, C. K. and Sprules, W. G., *J. Plankton Res.*, 1986, **8**, 79–90.
6. Francois, Y., *Bull. Soc. Zool. Fr.*, 1948, **74**, 232–239.
7. Bayly, C. C. E., *Crustaceana*, 1963, **6**, 75–80.
8. Altaff, K., Ph D thesis, University of Madras, 1990, p. 168.
9. Fleminger, A., *Mar. Biol.*, 1985, **88**, 273–294.
10. Hopkins, O. C. E., *J. Exp. Mar. Biol. Ecol.*, 1982, **60**, 91–102.
11. Rajendran, M., Ph D thesis, Madurai Kamaraj University, 1973.
12. Peter Gray, *Handbook of Basic Microtechnique*, McGraw Hill, New Delhi, 1964.

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