

Chicken embryos (ten day-old) were inoculated with 0.2 ml of virus suspension by chorio-allantoic route. The embryos showing death after 48 hr and those still surviving on the sixth day post-inoculation were chilled at 4°C. Embryos were harvested after overnight chilling. Chorio-allantoic membranes (CAMs) and embryos were examined for the presence of lesions, characteristic of infectious bursal disease virus (IBDV). CAMs were washed in phosphate buffer saline (PBS, pH 7.2) and 20% suspension (w/v) was prepared. This suspension was inoculated in another batch of embryos and ten serial passages were carried out in a similar way. To demonstrate the presence of viral entity at every passage level agar gel precipitation test (AGPT) was performed.

Egg infective dose (50%) (EID_{50}) of passage ten IBDV (IBDV/10) was determined³ and the neutralization test was performed by mixing equal volumes of 10 EID_{50} , 100 EID_{50} and 1000 EID_{50} /0.2 ml of the virus and the heat inactivated (56°C for 30 min) immune and normal serum to confirm the identity of virus. Virus-serum mixtures were incubated at 37°C for 1 hr and 0.2 ml was inoculated in embryonating eggs by CAM route.

The embryos showed 100% and 45% mortality after 72 hr in passage numbers one and two respectively. However, in the subsequent passages, no mortality was observed and hence the embryos were sacrificed on 6th day post-inoculation. The CAMs of dead and sacrificed embryos showed congestion, edema and thickening. Embryos showed curling after fifth passage. In the subsequent passages both curling and dwarfing were noticed. Thus the CAM route was successfully used for the serial passages of IBDV in chicken embryo as reported earlier⁴⁻⁶. The apparent reason for the absence of mortality after second passage is not fully understood. The route of inoculation is important in influencing embryo mortality^{5,7}, while the absence of mortality can be attributed to the propagation of the agent in fertile eggs from non-susceptible flock and the selection of wrong embryo materials for sub passages. Failure to cause embryo deaths in subsequent passages in the present study cannot be explained on this basis since: (i) there was no evidence of infection of IBD on the farms from which the eggs were utilized for the propagation of the agent, (ii) other characteristic lesions of IBDV such as congestion and edema of CAM, curling of embryo etc were noticed in the subsequent passages, (iii) CAMs were utilized for the serial passages which were shown to have higher titres than allantoamniotic fluids⁷, (iv) presence of viral entity was demonstrated at every passage level by AGPT and

(v) a titre of $10^{6.38}$ EID_{50} /0.2 ml was obtained at IBDV/10. It is therefore necessary to use specific pathogen-free eggs for such purposes.

In conclusion the findings suggest that the isolate was well adapted to the chicken embryo confirming the earlier reports^{4, 5, 8}.

The authors thankfully acknowledge Dr A. V. Manda and Dr B. D. Survashe for their keen interest in the present work.

20 August 1983; Revised 4 July 1984

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A NEW RECORD OF *DIAPHANOSOMA SENEGALENSIS* GAUTHIER, 1951 (CLADOCERA, SIDIDAE) FROM MADURAI, SOUTH INDIA.

K. VENKATARAMAN and S. KRISHNASWAMY,

*Department of Environmental Biology,
School of Biological Sciences,
Madurai Kamaraj University,
Madurai 625 021 India.*

ALTHOUGH Cladocera are among the commonest micro-crustaceans, a perusal of literature of this group shows that they are poorly known taxonomically throughout India¹⁻⁴. There is no comprehensive systematic study of the species of Cladocera in Tamil Nadu except that of a 'guide to the freshwater organisms'², which is a preliminary attempt to identify eight of the common genera occurring in Madurai. The present work was undertaken in 1979 to study the

taxonomy of Cladocera of southern Tamil Nadu based on intensive and extensive sampling and detailed study of all available species. Over 531 samples were collected from all types of habitats. Several females of *Diaphanosoma senegalensis* Gauthier were collected from the reddish-brown ponds of Madurai and Ramnad districts.

D. senegalensis Gauthier, 1951 (figure 1) Synonym: *D. hydrocephalus* Brehm, 1952, p. 139.

Size 2.00 mm, length greater than height; supra-ocular depression less prominent, dorsal margin hump-backed, brood pouch superior to cephalic margin; valve oblong, oval, posteroventral corner of the duplicature narrow with 12–15 long feathered setae. Head large with eye situated just above the ventral margin; antennular muscles well developed; antenna well developed, setae on antenna: 4–8/0–1–4; spines: 1–1/0–1–1; antenna ending just before the valve, postabdomen tapering at the distal region, dorsal margin stright with groups of prominent denticles and groups of lateral setae; claw with three long basal spines which increase in length towards distal end with seta on the ventral distal and concave margin.

Among the species of *Diaphanosoma* collected from

around Madurai, this is the largest, and it can easily be recognised by the presence of denticles on the posterior margin of the valve. The distinguishing characters of the species are: broad head with large eyes in the middle; brood pouch superior to the cephalic margin; well developed duplicature with feathered setae; presence of denticles on the posterior margin; presence of prominent spines on the distal ramus of the antennae and conspicuous lateral spines on the post abdomen. The posterior margin of the valve is stright, compared to that in other closely related species and the denticles increase in size towards dorsal end. Besides the lateral side of the post abdomen the claw is also armed with spines.

The maximum recorded size of *D. senegalensis* from Poull Bourgon (Senegal) is 1.74 mm. The present material contains specimens upto 2.00 mm length.

The specimens described by Brehm from India under the name *D. hydrocephalus* appear to be synonymus with the North African *D. senegalensis*. Though we have not seen Brehm's material, his illustration and description leave no doubt about the conspecificity of his specimens with *D. senegalensis*.

So far *D. senegalensis* has been reported from North Africa and North India. This is the first record of the occurrence of this species in South India.

The name *D. senegal* given by Gauthier seems to be inappropriate according to International Rules of Zoological Nomenclature. So we have emended the name to *Diaphanosoma senegalensis*.

KV is thankful to CSIR for financial assistance.

16 December 1983; Revised 23 July 1984

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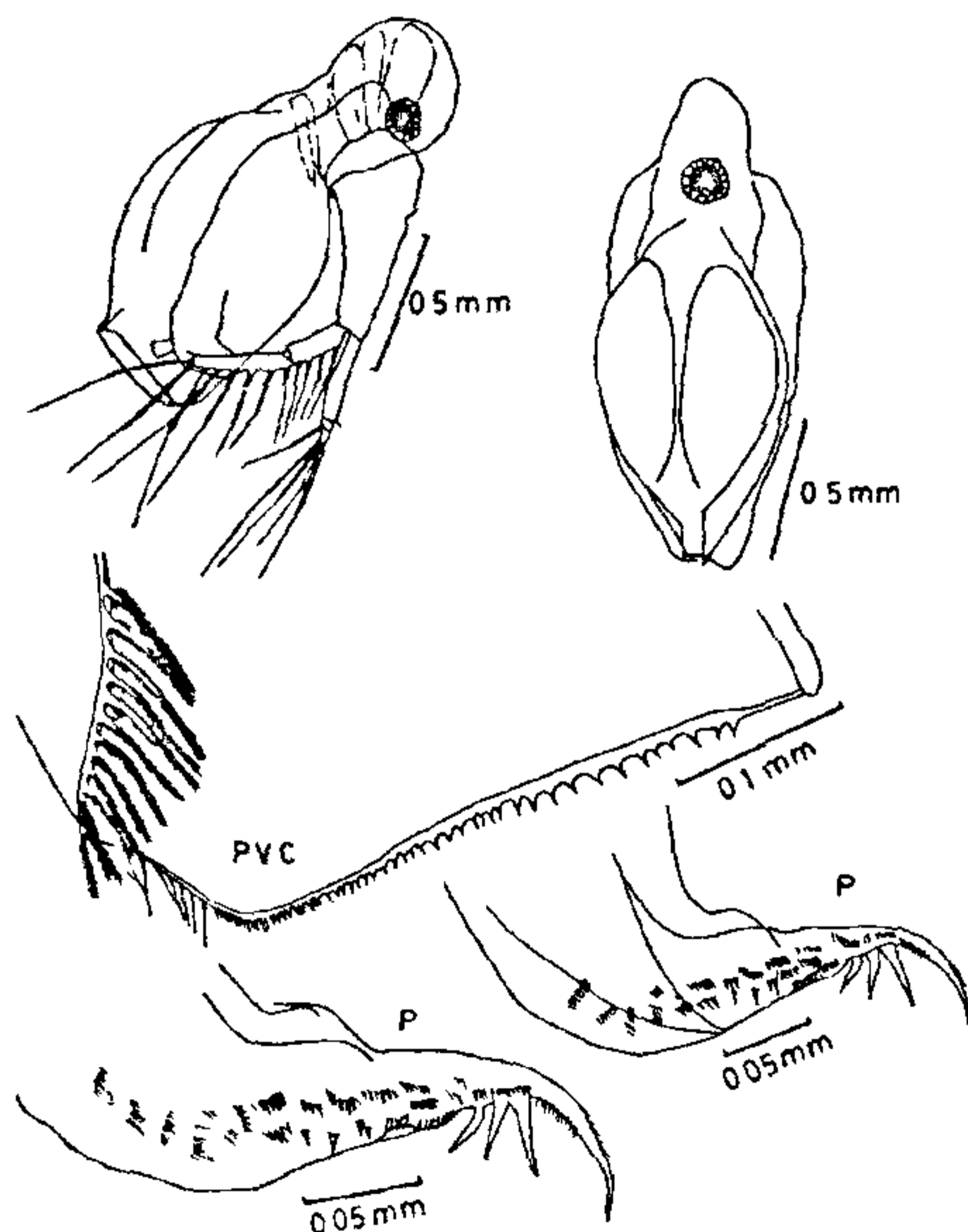


Figure 1. *Diaphanosoma senegalensis*, female: PVC—posteroventral corner; P—postabdomen.