

## Chromosome biology of the scorpion *Palamnaeus* sp.

M R RAJASEKARASETTY, N V ASWATHANARAYANA and  
K R KUMARASWAMY

Department of Zoology, University of Mysore, Manasa Gangotri, Mysore 570 006

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**Abstract.** The chromosomes of the male scorpion *Palamnaeus* sp. has been analysed making use of the Colchicine-hypotonic-suspension technique. The diploid number has been found to be 62. The presence of clear constrictions on the spermatogonial metaphase chromosomes is noticed. The occurrence of diplotene-diakinesis, chiasmata and translocations have been encountered. The nature of the kinetochore is discussed. It is opined that the chromosomes of this scorpion are apparently monocentric (represented by constrictions on chromosomes) but behaving as holocentric.

**Keywords.** *Palamnaeus* sp.; constrictions; holocentric; scorpion.

### 1. Introduction

Even though the cytology of scorpions is well documented, the architecture of the chromosomes, especially the nature of the kinetochore, has been highly controversial (Piza 1943, 1947, 1948, 1950, 1957; Brieger and Graner 1943; Brieger and Kerr 1949; Sharma and Joneja 1959; Sharma *et al* 1959; Srivastava and Agarwal 1961; Guenin 1957, 1961; Venkatanarasimhaiah and Rajasekarasetty 1964, 1965; Venkatanarasimhaiah 1965). All the previous workers studied the chromosomes in sectioned and squash materials. This study is an attempt to re-examine the issue using a different technique.

### 2. Material and methods

The males of the scorpion *Palamnaeus* sp. collected from the environs of Manasa Gangotri, Mysore, formed the material for the present investigations. They were injected abdominally with 0.2 ml of 0.05% colchicine and sacrificed after 2 hr. The testes were dissected out and treated with 0.9% sodium citrate solution for 30 min and fixed in freshly prepared 1 : 3 acetic acid/methanol. The chromosome preparations were made by the suspension technique, flame-dried and stained with Giemsa.

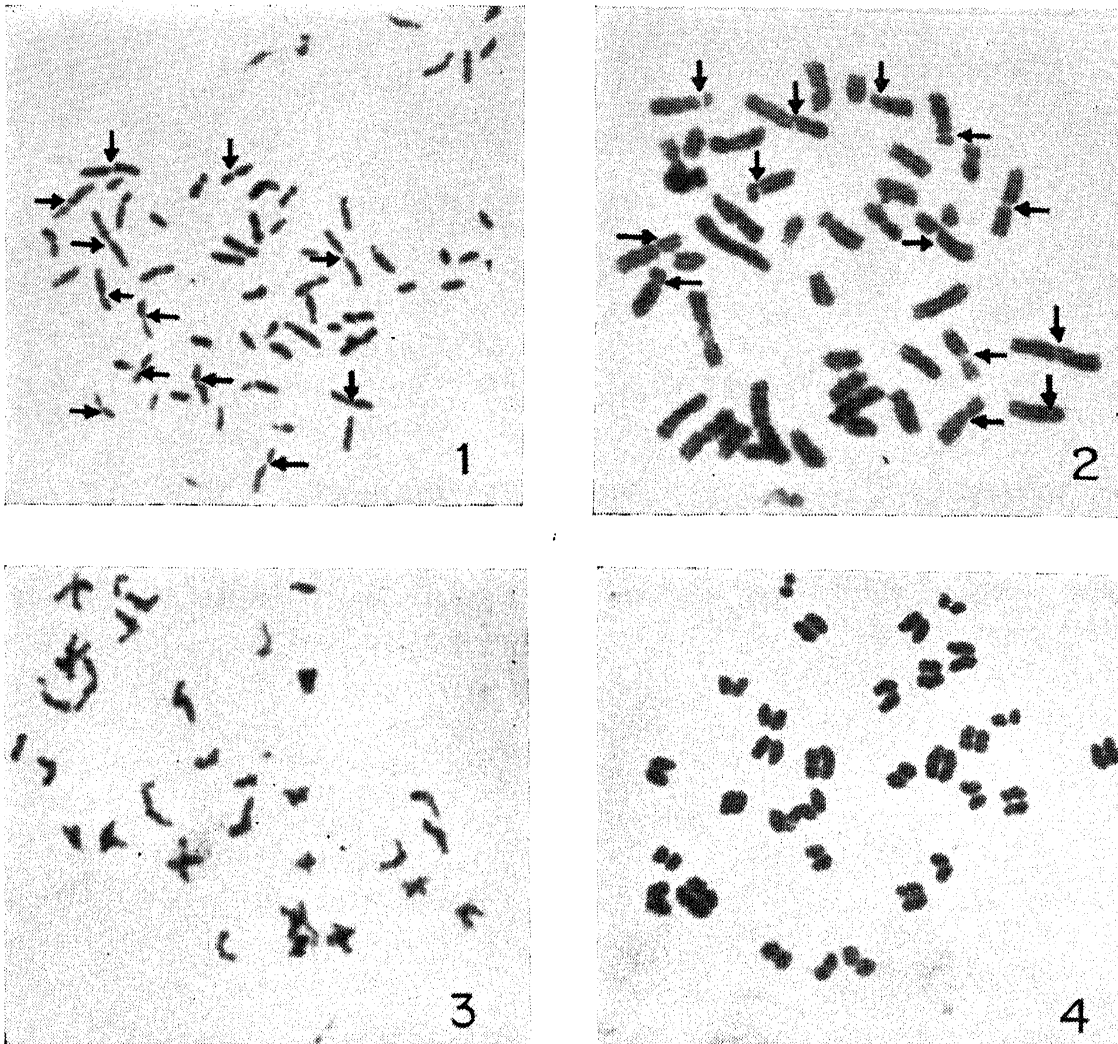
### 3. Observations

The genome of this scorpion is characterised by the presence of a diploid number of 62 chromosomes as scored from many spermatogonial metaphases (figure 1). The chromosomes fall into large, medium and small sizes and possess one distinct constriction in each of the two chromatids of metaphase chromosomes at the median, submedian and subterminal positions (figure 2). Not more than one clear constriction representing very likely the primary one has been seen in a chromosome. The two chromatids of each chromosome lie parallel to each other except at the primary constriction region. The occurrence of one chiasma per bivalent in the diplotene stage is mostly clear (figure 3). Further tightening of the coils, increase in the girth, terminalisation of chiasmata and parallel disposition of the homologues result in diakinesis (figure 4). It is very interesting to note that translocation crosses and open-chains have been encountered during the cytological analysis. The interchanges involved 6 or 8 chromosomes (figures 5 and 6). The occurrence of interchanges in this population is being reported for the first time. The anaphases are characterised by the presence of V-shaped and rod-shaped configurations which lie parallel to the main spindle axis (exhibiting axial orientation) (figures 7 and 8).

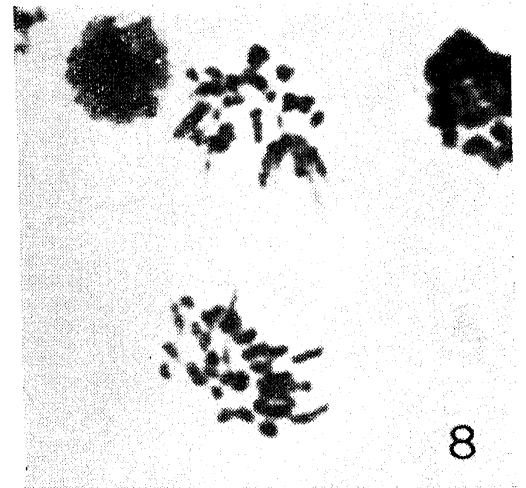
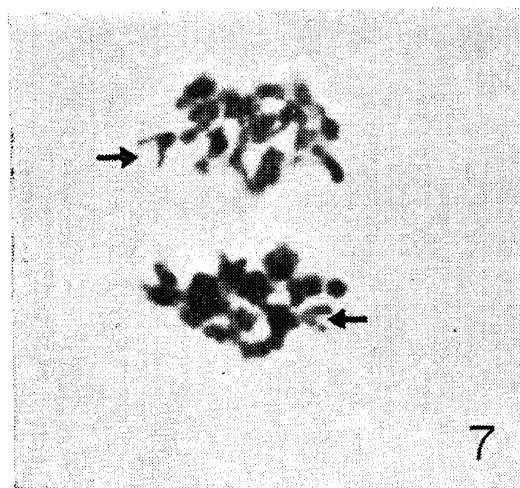
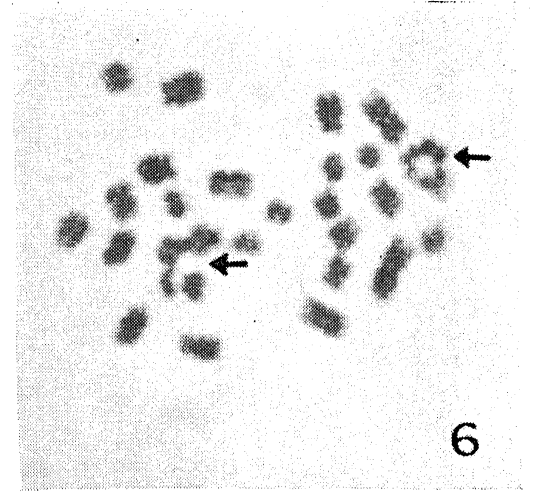
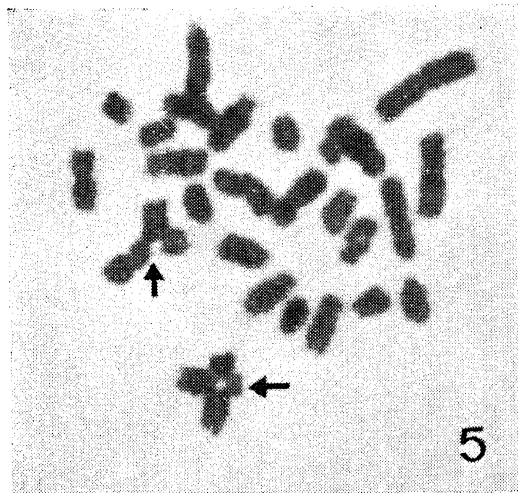
### 4. Discussion

There are many divergent views with regard to the presence of diplotene-diakinesis, the formation of chiasmata and whether the chromosomes are holokinetic or monocentric. In *Tityus bahiensis* (Piza 1943), *Tityus mattogrossensis* (Piza 1947), *Rhopalurus* (Piza 1957), and in three Indian Buthids (Sharma *et al* 1959) the absence of the formation of chiasma and diplotene-diakinesis were reported. On the other hand, in *Palamnaeus longimanus* (Srivastava and Agarwal 1961) the existence of diplotene-diakinesis stage, with no chiasma formation, was claimed. The occurrence of diplotene-diakinesis and chiasma formation was noticed in *Buthus occitanus* (Guenin 1961). It was claimed by Brieger and Graner (1943) that they have seen some configurations suggestive of chiasma formation in *Tityus* sp. But Piza (1943) has pointed out that the homologues simply remain paired throughout their length from pachytene upto the beginning of anaphase-I. Venkatanarasimhaiah and Rajasekarasetty (1964) working on the chromosomes of *Palamnaeus gravimanus* reported the presence of diplotene-diakinesis stage and chiasma formation. In the present studies also on *Palamnaeus* sp. the authors have clearly seen the occurrence of diplotene-diakinesis and chiasma formation. The configurations of diplotene bivalents are characteristic and reminiscent of the configurations noticed in grasshopper meiosis.

The translocations reported by Piza (1943, 1947, 1948, 1950) in various Brazilian species such as *Tityus* and *Isometrus* are very interesting, since certain individuals are heterozygous for several translocations, leading to the formation of multiple rings at meiosis, similar to those of the plant *Oenothera* (White 1973). Piza has also shown the occurrence of single, two or multiple cross-shaped configurations at meiosis involving 4, 5, 7 and 8 chromosomes and thereby indicating the presence of multiple translocations. Sharma *et al* (1959) have also reported the occurrence of translocation heterozygosity in two species of Indian



**Figures 1-4.** 1. Spermatogonial metaphase chromosomes ( $2n\delta = 62$ ) showing constrictions (arrows). 2. An incomplete spermatogonial metaphase plate showing distinct constrictions on the chromosomes at median and submedian positions (arrows) and clearly exhibiting parallel disposition of the chromatids. 3. Diplotene bivalents with distinct chiasmata. 4. Diakinesis with parallelly disposed homologues.



**Figures 5-8.** 5. Diakinesis showing triangular (involving 6 chromosomes—arrow) and cross-shaped (involving 8 chromosomes—arrow) configurations of interchanges. 6. Diakinesis having translocation open-chain (arrow). 7. Anaphase-I showing V-shaped configurations (arrows) with axial orientation. 8. Anaphase-II having rod-shaped configurations with axial orientation.

Buthid scorpions. Venkatanarasimhaiah and Rajasekarasetty (1964) have not reported the occurrence of chromosomal interchanges in *Palamnaeus gravimanus*. In the present observations on the chromosomes of the scorpion *Palamnaeus* sp. the authors have encountered chromosomal interchanges with the formation of ring and open-chain configurations involving 6 or 8 chromosomes.

The position of the kinetochore in the scorpion chromosomes has been a subject of conjecture and debate and various authors have expressed different views. Brieger and Kerr (1949) have opined that the scorpion chromosomes should be considered as holocentric. Radiation studies of Rhoades and Kerr (1949) have suggested the diffused or polycentric nature of scorpion chromosomes. Although in some non-buthid scorpions such as *Opisthacanthus* (Wilson 1931) the chromosomes seem to be monocentric, those of *Tityus* and *Isometrus* (Piza 1943) do not show any constrictions which might indicate the presence of an individual centromere. He also pointed out that at both mitotic and meiotic anaphases the daughter chromosomes remain straight and parallel to the equatorial plate as if they were attached to the spindle along the whole length. In his later works he put forward the view that one kinetochore is present at each end of the chromosome. Piza (1947) has reported the monocentric nature of the chromosomes in *Bothriurus* sp. The chromosomes of *Pandinus imperator* and *Buthus occitanus* have been described as metacentric and acrocentric (Guenin 1957, 1961) based on the presence of constrictions on the chromosomes. Sharma *et al* (1959) have reported the diffused nature of kinetochore in the Indian Buthids. Based on the fact that the long axis of the anaphase chromosomes is parallel to the spindle axis and the presence of constrictions on the chromosomes, Srivastava and Agarwal (1961) have claimed that the chromosomes of *Palamnaeus longimanus* are monocentric. Venkatanarasimhaiah and Rajasekarasetty (1965) have considered the chromosomes of *Heterometrus scaber* and *Heterometrus fulvipes* to be monocentric and acrocentric based on the fact that the long axes of the anaphase-I chromosomes lie parallel to the main axis of the spindle. In the colchicine injected *Palamnaeus swammerdami* they have shown the presence of subterminal, submetacentric and metacentric constrictions at prometaphase-I. Based on the orientation of chromosomes during metaphase-I and anaphase-I, they have come to the conclusion that the chromosomes of *Buthus tamulus* are monocentric, bicentric and holokinetic. In all these genera and species, the figures do not show clear non-staining definite areas which connote the centromeric regions or the lack of it. They are based on the topography of the chromosomes in relation to the spindle axis.

Using the improved technique, the photographs of the scorpion chromosomes show very clearly the non-staining constrictions at median, submedian and subterminal positions in the spermatogonial metaphases in *Palamnaeus* sp. under study. Even though the diplotene configurations in the present studies are similar to the diplotene configurations of the true monocentric chromosomes of grasshoppers, such configurations are also noticed in the holocentric chromosomes of bugs and earwigs. This is a point to be considered. Further, the parallel disposition of the chromatids noticed in the spermatogonial metaphase plates of *Palamnaeus* sp. does not show the characteristic "lever" mechanism noticed in the true monocentric chromosomes of grasshoppers and mammals where the two chromatids open out from the region of the kinetochore, specially in those of colchicine-treated chromosomes. But in the case of the scorpion under study, the

two chromatids lie parallel during spermatogonial metaphase and so also the homologues during diakinesis or metaphase-I. The possibility that these constrictions may be despiralised regions was given up because of the consistent nature of these regions. Hence it is believed that the region of the constriction (kinetochore) must have lost the capacity to function as kinetochore and there must have been an attempt for the acquisition of neocentric activity (reflected in some sort of attraction or force between the chromatids to keep them parallelly disposed) which became a permanent feature. Therefore it is our opinion that the chromosomes of this scorpion *Palamnaeus* sp. must be apparently monocentric (represented by constrictions on chromosomes) but behaving as holocentric.

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