Uttar Pradesh J. Zool. 27(3) : 325-329, 2007 ISSN 0256-971X

THE KARYOTYPES OF POUCHED AMPHISTOMES

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The karyotypes of the three amphistomes, *Gastrothylax crumenifer* (Creplin, 1847) Poirier, 1883, *Fischoederius cobboldi* (Poirier, 1883) Stiles and Goldberger, 1910 and *Fischoederius elongates* (Poirier, 1883) Stiles and Goldberger, 1910 from ruminants were studied using the squash and flame drying techniques. Except *F. elongates* (2n = 16) other two species demonstrate 2n = 18 chromosomes. Meiotic configurations were also studied to substantiate chromosome numbers. The results are discussed in terms of evolutionary relationship of morphologically well defined amphistomes.

Key words : Karyotypes, *Gastrothylax crumenifer, Fischoederius cobboldi, F. elongatus, Carmyerius* trematode species.

INTRODUCTION

The amphistomes are the digenetic trematodes found as endoparasites in the digestive tracts, liver and bile ducts of many vertebrates. These amphistomes belong to the varied genera and subfamilies that fall under the family Paramphistomidae. The genus *Fischoederius* and *Gastrothylax* occurs in the rumen of cattle and other Bovidae. *Fischoederius* measures 10-20 mm long and the breadth is about one quarter of the length. It closely resembles *Gastrothylax*, but one testis lies dorsal to the other and the uterus runs forward in the midline. The intestinal caecae are not widely separated and end a short distance behind the middle of the body. *F. cobboldi* differs from the *F. elongates* in being only 8-10 mm long, while the intestinal end at the posterior border of the posterior testis. The taxonomy of the paramphistomes is complex. It is discussed with the relevant literature by Nasmark (1937), Dawes (1946), Edurdo (1982) and Gupta (1993). The three genera *viz. Gastrothylax, Fischoederius* and *Carmyerius* are characterized by the presence of a ventral pouch which occupies almost the whole of the ventral portion of the body. Yamaguti (1971) recognized 33 subfamilies under the family Paramphistomidae. The above genera are grouped under subfamily Gastrothylacinae. The diploid number of chromosomes in amphistomes range from 12-22 (Barsiene, 1993; Subramanyam & Venkat Reddy, 1977). Our knowledge on amphistome cytogenetics is rather limited. An attempt is made here to compare the karyotypes of four pouched amphistomes to find out whether there are any common chromosomes features which could be used to show interrelationships.

MATERIALS AND METHODS

Adult amphistomes collected from Cattle at Amberpet abattoir, Hyderabad. Parasites were put in saline and transported to the laboratory. In the case of *Gastrothylax* testes were dissected pooled in a hypotonic solution and thoroughly minced to make fine suspension. Centrifugation was carried out at 1000 r.p.m. for 5 min. The supernatant was removed and replaced by 1:3 acetic acid absolute methyl alcohol. Procedure repeated thrice and preparations were made by flame drying technique (Subramanyam & Venkat Reddy, 1976).

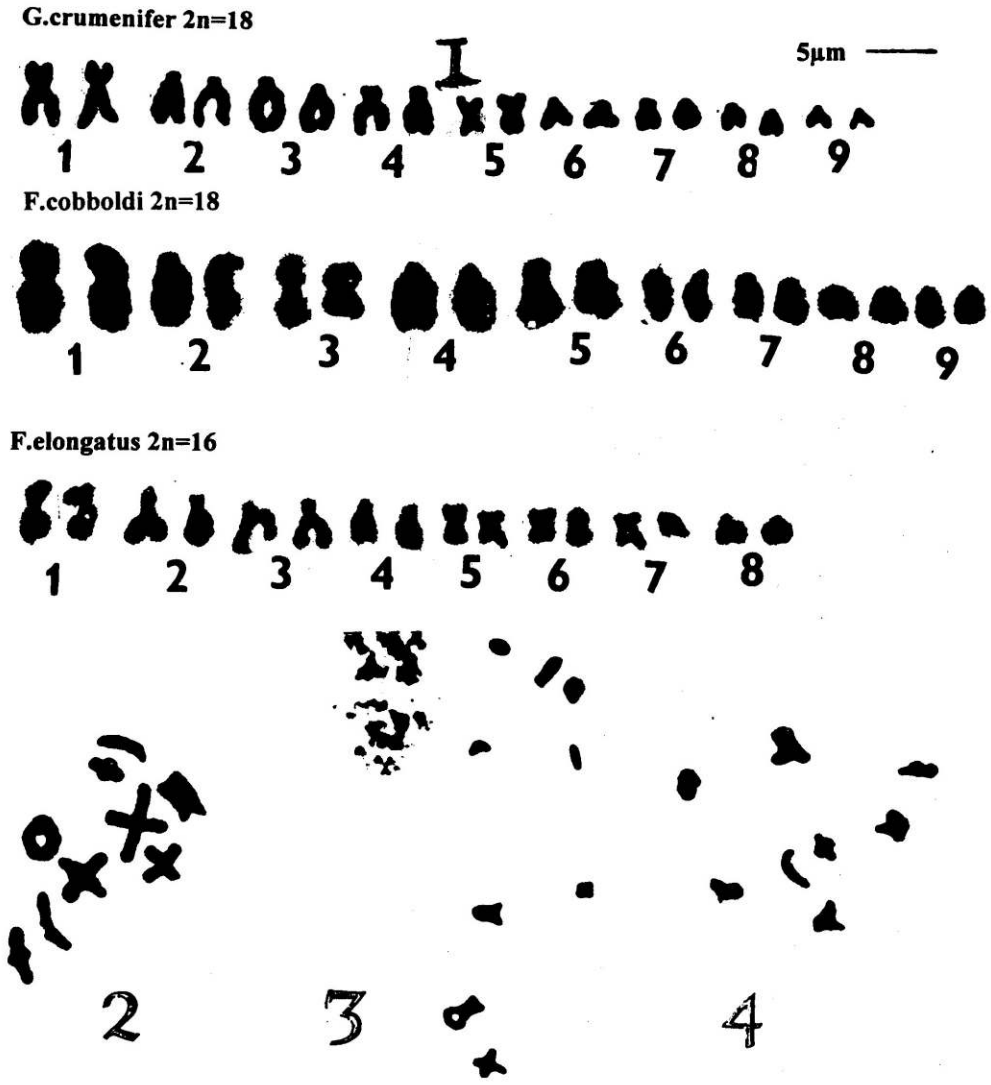
The method of processing *Fischoederius* material was essentially the same as described elsewhere (Venkat Reddy & Subramanyam, 1971) chromosome measurements were made from ten well spread spermatogonial metaphase pictures. The karyotype description and nomenclature for centromeric position follow the system of Levan et al. (1964) and White (1973).

RESULTS AND DISCUSSION

Dhingra (1955) reported the haploid and diploid chromosome number of *G. crumenifer* as 07n and 14n, respectively from sectioned material and attempted to classify them to some extent on the basis of their morphologies. In the brief report on chromsome members of some numbers of the family Paramphistomidae, Sharma *et al.* (1968) only mentioned 18 as the diploid number of *G.crumenifer* Romanenko (1974) identified only meta-and submetacentric chromosomes. The spermatogonial divisions from flame dried preparations reveal eighteen as the diploid number of *G. crumenifer*. The karyotype constructed from the enlarged chromosomes clearly show one heterobrachial, one isobrachial five subtelocentric and two telocentric chromosome pairs. An analysis of a large number of meiotic cells has helped in the confirming the above number by the presence of nine bivalents in metaphase I (Fig. 2).

Jha (1975) reported nine bivalents at metaphase I and nine dyads in metaphase II from meiotic stages of *F. cobboldi* and concluded the diploid number to be eighteen. Rhee (1988) reported 2n=18 and identified four pairs metacentric and five pairs of submetacentric chromosomes. This parasite has therefore been reinvestigated for its cytology. The author established 2n=18 without any ambiguity. Karyotype of *F. cobboldi* represents one hetero-brachial metacentric, one isobrachial metacentric one submetacentric and six telocentric chromosome pairs. The number reported by the author agrees with the diploid counts given by the Jha (1975) and Rhee (1988) for *F. cobboldi*. The parasite has the highest number of telocentric chromosomes with in the family Paramphistomidae (Subramanyam & Venkat Reddy, 1977). This could be effectively used as a means of identifying the parasite on cytological grounds.

As regards *F. elongates* the diploid number was reported as twenty two by Jha (1975). His observations were based exclusively on meiotic cells from squashes and paraffin sections respectively. It is not known how he classified the twenty-two chromosomes of *F. elongates* to be made up of four metacentric and eighteen acrocentric chromosomes. He has mentioned metaphase plates of spermatogonial second metaphase as well as first metaphase plates, pachytene and diplotene stages were used for locating the centromeres in order to determine the morphology of chromosomes. However, no karyotype nor idiogram were provided to substantiate these observations. After reinvestigation this has been correctly established as 2n=16 for *F.elongatus* (Venkat Reddy & Subramanyam, 1983). The karyotype of *F. elongates* constitutes two isobrachial one heterobrachial and five submetacentric chromosome pairs. It is interesting to note the complete absence of telocentrics which are prevalent in other related species (Table I). Karyotype of *F .elongatus* is unique among known pouched amphistomes.



Figs. 1-4 : *C. crumenifer*, *F. cobboldi* and *F. elongates*. **1.** Karyotypes; **2, 3 & 4.** Metaphase I.

Ashour (1995) described cytology of *C.gregarius* from Egyptian ruminants. He classified diploid number (2n=18) as one submeta, three subtelocentric five acrocentric chromosome pairs. A careful perusal of Table I show close similarity with *F. cobboldi* with reference to occurrence of telocentric chromosomes but differs with other species with reference to complete absence of metacentric chromosomes.

The diploid number of chromosomes varies from 12-22 in the family Paramphistomidae (Basiene, 1993). Subfamily Gastrothylacinae represents four morphologically well defined species *viz. G. crumenifer*, *F. cobboldi*, *F. elongates* and *C. gregarius*. An attempt is made here to compare the karyotypes of above species to find out whether there are any common

Table I : Quantitative characteristics of spermatogonial chromosomes of four amphistomes.

Chromosome pair	1	2	3	4	5	6	7	8	9
G. crumchifer 2n = 18									
S.a	1.8	0.8	0.5	1.1	1.4	0.7	0.6	1.7	1.6
La	2.9	2.9	3.2	2.3	1.4	1.6	1.6		
R.I.	18.01	14.18	14.18	13.03	10.73	8.81	8.43	6.51	6.13
A.r	1.61	3.63	6.40	2.09	1.00	2.29	2.67		
C.i	38.30	21.62	13.51	32.35	50	30.43	27.27	0.0	0.0
C.c	HM	ST	ST	SM	IM	SM	SM	T	T
F. cobboldi 2n = 18									
S.a	2.00	3.8	1.6	3.4	0.8	2.9	2.1	1.9	1.9
La	2.8		1.8		2.2				
R.I.	17.65	13.97	12.50	12.50	11.03	10.66	7.72	6.98	6.98
A.r	1.40		1.12		2.75				
C.i	41.67	0.0	47.06	0.0	26.67	0.0	0.0	0.0	0.0
C.c	HM	T	IM	T	SM	T	T	T	T
F. elongates 2n = 18									
S.a	1.7	0.9	0.9	1.0	1.4	1.2	0.9	0.6	
La	4.5	3.1	2.9	2.7	1.4	1.2	1.4	1.6	
R.I.	22.63	14.60	13.87	13.50	10.22	8.76	8.39		
A.r	2.65	3.44	3.22	2.70	1.00	1.00	1.55		
C.i	27.42	22.50	23.68	27.03	50.00	50.00	39.13		
C.c	SM	SM	ST	SM	IM	IM	HM	SM	
C. gregarious 2n = 18*									
R.I.	17.78	17.79	16.89	12.04	9.75	7.45	6.69	6.3	5.54
C.I.	0.37	0.25	0.18	0.33					
C.c	SM	ST	ST	ST	A	A	A	A	A

* : Data from Ashour *et al.* (1995)

S.a. : Short arm; L.a. : Long arm; C.c : Chromosome classification; IM : Isobrachial metacentric; HM : Heterobrachial metacentric; SM : Submetacentric; ST : Subtelocentric; A : Acrocentric; T : Telocentric.

RI (Relative length) = Length of chromosome x 100 / Haploid genome
A.r (Arm ratio) = Length of Long arm / Length of short arm
C.I. (Centromeric Index) = Length of short arm x 100 / Length of whole chromosome

features which could be used to show interrelationships. Quantitative characteristics such as relative length, arm ratio and centromeric index of chromosomes is a invaluable data concerning the origin and nature of the chromosomal differences that exists in related species. The karyotype is a fundamental feature of a species as Stebbins (1950) states "the chromosomes because they are bearers of hereditary factors should be considered as some what more basic than other structures on which relationship is based". Majority of speciating events are accompanied by karyotypic alterations like chromosomal rearrangements and initiated divergence (White, 1973). Chang & Carson (1985) opined that in most species the fixation of particular type of karyotype is likely to be merely an incidental accompaniment of small population effects and forced selection for reorganiza-

tion as the species is formed. Another possible mechanism about the increase in chromosome number could be aneuploidy However, the cytogenetics of pouched amphistomes based on recent techniques is still meager, extensive use of karyotype data to establish interrelationship must await further studies on unexplored species in various genera of amphistomes. It is worthwhile to study other unexplored species of pouched amphistomes for their cytology. Further, it is necessary to employ various chromosome banding techniques to conclude about their homology and common ancestry.

ACKNOWLDEGEMENTS

The author is grateful to The Management, Vishwambhara Educational Society, for providing facilities and encouragement.

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