Mitotic karyotype of the tropical freshwater crayfish *Procambarus* (*Austrocambarus*) *llamasi* (Decapoda: Cambaridae)

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Abstract: In Mexico, the biology of *Procambarus* has been more studied than the biology of other Cambarids because of its diversity and potential use in aquaculture. We determined the karyotype of the Mexican tropical freshwater crayfish *Procambarus* (*Austrocambarus*) *llamasi* from 189 metaphase spreads from gill tissues of 17 adults. They had 98-120 chromosomes (mode 2n=120 chromosomes). There are 60 pairs of monoarm, telocentric chromosomes. Sex chromosomes were not detected and we propose that the *P. llamasi* karyotype can be used to distinguish this species from other Mexican crayfish. Additionally, we suggest using karyological data in aquaculture and conservation biology. Rev. Biol. Trop. 58 (2): 655-662. Epub 2010 June 02.

Key words: chromosome number, karyotype, tropical crayfish, *Procambarus* (*Austrocambarus*) *llamasi.*

There are three genera and about 50 species of cambarids in Mexico: *Procambarus* with 39 species, *Cambarellus* with 10 and *Orconectes* with only one species (Villalobos-Figueroa 1983). In that country, the biology of *Procambarus* has been more studied than the biology of other Cambarids because of its diversity and potential use in aquaculture (Villalobos-Figueroa 1983, Diupotex-Chong *et al*. 1997, Rodríguez-Serna *et al*. 2000, 2002).

Since Villalobos-Figueroa (1983) reported the endemic distribution of the freshwater crayfish *Procambarus* (*Austrocambarus*) *llamasi* to the south-east region of Mexico, several studies have been done on its biology (Morales & Bosada 1987), ecology (Morales *et al*. 1987, Villalobos-Hiriart *et al*. 1993), reproductive behavior (Mendoza 1994), reproduction (Rodríguez-Serna *et al*. 2000) and geographic distribution (Rodríguez-Serna *et al*. 2002). However, studies on basic genetics like chromosome structure for karyotype determination are still rare, with exception of a study on *P. diugeti* which revealed a chromosome number of 2n=102 chromosomes (Diupotex-Chong *et al*. 1997).

In general, cytogenetics studies of crustaceans are relatively few and very difficult to perform because their chromosome numbers are large (Niiyama 1962, Roberts 1969, Mittal & Dhall 1971, Campos-Ramos 1997, Dumas & Campos-Ramos 1999, Zhang *et al*. 2003, Lee *et al*. 2004), chromosomes are small and theirs shapes are very variable including metacentric, submetacentric, and acrocentric chromosomes (Tan *et al*. 2004, Salema & Heino 1990, Damrongphol *et al*. 1991). Such distinctiveness makes them difficult to karyotype in comparison with the chromosomes of insects and some vertebrate species (White 1973).

Early karyological studies have provided basic information on the number, size, and morphology of chromosomes which is an important prerequisite to the use of techniques for set chromosome manipulations in fishes (Arai 2001, Hulata 2001), marine crustaceans, such as the white shrimp *Litopenaeus vannammei* (Dumas & Campos-Ramos 1999), and the Chinese shrimp *Fenneropenaeus chinensis* (Zhang *et al*. 2003), as well as in marine bivalves such as the Japanese oyster *Crassostrea gigas* (Allen *et al*. 1989). Consequently, information on the basic genetics of the tropical crayfish *P. llamasi* is necessary not only to reinforce its potential for aquaculture, but also for genetic improvements and conservation. The main aim of this study was to describe the mitotic chromosome number and karyotype of the tropical freshwater crayfish *P. llamasi* that inhabits the Grijalva River in Tabasco, southern of México.

MATERIALS AND METHODS

Sampling site and crayfish maintenance: Seven adult females and ten males of *P. llamasi* ranging from 5.5-14.0cm of total length were used. Crayfish specimens were collected from the artificial creeks along the Academic Division for Biological Sciences of the Juarez University Autonomous of Tabasco (DACBIOL-UJAT); specimens frequently invade during the raining season (October-November) when the Grijalva River fills up with flood waters. Collected specimens were reared in the laboratory and fed daily with shrimp pellets throughout this study.

Cytological procedure and preparation of chromosome slides: crayfish specimens were processed following the procedure by Lakra *et al* (1997) and Arias-Rodriguez *et al*. (2007, 2008) with slight modifications as follows: Specimens were incubated for five hours in a well-aerated plastic bottle containing 10ml of freshwater that were mixed with 1.0ml of 1.0% colchicine solution. Then crayfish were sacrificed and gill tissue dissected out. Minced gill tissues were kept in hypotonic treatment with 1.0% sodium citrate for two hours at room temperature. Well swollen tissues were fixed in 4:1 fresh fixative solution made by chilled methanol (4°C) and glacial acetic acid. Three changes were essential at the interval of 15 min to give appropriated fixation of the cytological tissue by a refrigerated (4°C) centrifugation at 3000r.p.m for 10min. The tissues were kept then at 4°C for about one month.

Chromosome slides were prepared from the fixed tissues following the methods by Arias-Rodriguez *et al*. (2008) after adoption of the technique by Lakra *et al*. (1997) technique**.** The chromosome slides were stained for about 30min in giemsa diluted at 10.0% with phosphate buffer (pH=7.0); and washed with distilled water before letting air-dry at room temperatures for 24 hours.

Microscopic analyses, karyotype assembling and classification: Well-spread mitotic metaphases were photographed using a Zeiss Axiostart plus microscope at 100X of magnifications by a coupled digital camera Sony Cybershot (DSC-W30). Diploid chromosome (2n) number or modal chromosome number was calculated on the base of the most frequently occurring measurement by counting the metaphases chromosomes.

Four of the best well spread mitotic metaphases were employed to assemble the most parsimonious karyotype of the species based on shape and measurements on chromosomes arms p (short arm) using Photoshop 6.0 (Adobe®). The data-base was collected in the Microsoft® Excel-2003 for analysis and classification of karyotype formula as recommended by Levan *et al.* (1964). The ideogram was drawing on the basis of the averaged length of each homologous chromosome pair (p) and their centromeric position.

RESULTS

A total of 189 well-spread metaphases were scored from 70 analyzed chromosome slides from 17 adult specimens of the tropical crayfish *P. llamasi*. The mitotic counts ranged

from 98 (2.6%) to 120 (67.7%) chromosome elements per diploid metaphase (Fig. 1). Basic statistical analysis indicated that modal chromosome number is 2n=120 chromosome elements found in 67.7% of the 189 examined metaphases (Fig. 1).

A typical diploid mitotic chromosome spread of the tropical crayfish *P. llamasi* is shown in figure 2-A, and the most parsimonious karyotype of the species in figure 2-B. The average size of the 60 pairs of telocentric (T) monoarm homologous chromosomes that comprise the karyotype of *P. llamasi* varied from the biggest pair one with 6.45±0.0 µm to the smallest pair sixty with 2.15±0.30 µm (Table 1, Fig. 2). The chromosome formula proposed for *P. llamasi* is 2n=120 telocentric (T) chromosomes (Table 1, Fig. 2). We did not detect any sex chromosomes (Fig. 2).

DISCUSSION

In cytogenetic studies, rapid growing tissues are required to obtain a large number of metaphase chromosomes spreads. Several karyological studies on crustacean have been performed from male gonad tissue (Murofushi & Deguchi 1983, Chow *et al*. 1990, Chavez-Justo *et al*. 1991, Damrongphol 1991, Xiang *et al*. 1994, Lakra *et al*. 1997, Tan *et al*. 2004). However, there are no available reports on karyotypes obtained from gill tissue of freshwater crayfish although the analysis of mitotic chromosomes provides a good tool to obtain the modal diploid chromosome number.

The mitotic procedure employed in this study allowed the establishment of the typical karyotype of *P. llamasi* that was characterized by 2n=120 monoarm chromosomes. The karyotype structure of this species shares some chromosomal similarities like variation

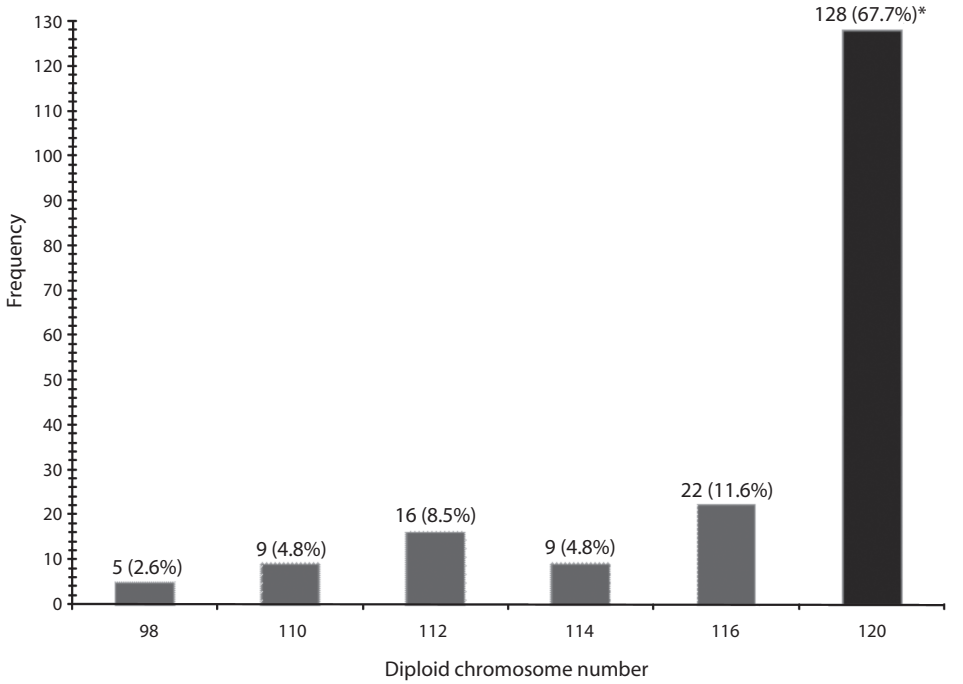

Fig. 1. Count distribution of chromosomes elements from gill tissue and diploid modal number (*) of 2n=120 chromosomes in *P. llamasi*.

TABLE 1

Length, relative length by chromosome pairs, and descriptive statistics for the karyotype of the tropical freshwater crayfish P. llamasi

Chromosome pairs	Averaged length in µm of q±SD	Averaged relative length of q±SD	Classification
1	6.45±0.00	2.53±0.00	T
2	6.45±0.00	2.53±0.00	T
3	6.24±0.30	2.45±1.16	T
4	6.02±0.00	2.36±0.00	T
5	6.02±0.00	2.36±0.00	T
6	5.59±0.61	2.19±2.33	T
7	5.38±0.30	2.11±1.16	T
8	5.38±0.30	2.11±1.16	T
9	5.16±0.61	2.02±2.33	T
10	5.16±0.61	2.02±2.33	T
11	5.16±0.61	2.02±2.33	T
12	5.16±0.61	2.02±2.33	T
13	5.16±0.61	2.02±2.33	T
14	5.16±0.61	2.02±2.33	T
15	4.95±0.30	1.94±1.16	T
16	4.95±0.30	1.94±1.16	T
17	4.84±0.46	1.90±1.74	T
18	4.73±0.61	1.85±2.33	T
19	4.73±0.61	1.85±2.33	T
20	4.73±0.61	1.85±2.33	T
21	4.73±0.61	1.85±2.33	T
22	4.73±0.61	1.85±2.33	T
23	4.62±0.46	1.81±1.74	T
24	4.52±0.30	1.77±1.16	T
25	4.52±0.30	1.77±1.16	T
26	4.52±0.30	1.77±1.16	T
27	4.41±0.46	1.73±1.74	T
28	4.30±0.61	1.69±2.33	T
29	4.30±0.61	1.69±2.33	T
30	4.30±0.61	1.69±2.33	T
31	4.30±0.61	1.69±2.33	T
32	4.30±0.61	1.69±2.33	T
33	4.30±0.61	1.69±2.33	T
34	4.19±0.46	1.64±1.74	T
35	4.09±0.30	1.60±1.16	T
36	4.09±0.30	1.60±1.16	T
37	4.09±0.30	1.60±1.16	T
38	3.87±0.61	1.52±2.33	T
39	3.87±0.61	1.52±2.33	T
40	3.87±0.61	1.52±2.33	T
41	3.87±0.61	1.52±2.33	T
42	3.66±0.30	1.43±1.16	T
43	3.66±0.30	1.43±1.16	T
44	3.66±0.30	1.43±1.16	T

TABLE 1
Length, relative length by chromosome pairs, and descriptive statistics for the karyotype of the tropical freshwater crayfish P. llamasi

Chromosome pairs	Averaged length in µm of q±SD	Averaged relative length of q±SD	Classification
45	3.44±0.61	1.35±2.33	T
46	3.44±0.61	1.35±2.33	T
47	3.44±0.61	1.35±2.33	T
48	3.44±0.61	1.35±2.33	T
49	3.23±0.30	1.26±1.16	T
50	3.23±0.30	1.26±1.16	T
51	3.01±0.61	1.18±2.33	T
52	3.01±0.61	1.18±2.33	T
53	3.01±0.61	1.18±2.33	T
54	3.01±0.30	1.18±2.33	T
55	2.80±0.30	1.10±1.16	T
56	2.80±0.30	1.10±1.16	T
57	2.58±0.30	1.01±2.33	T
58	2.15±0.30	0.84±0.00	T
59	2.15±0.30	0.84±0.00	T
60	2.15±0.30	0.84±0.00	T

q:short arm, SD:standard deviation, T:telocentric chromosome according to Levan *et al*. 1964.

in chromosome number, variation in size and shape as in other crustaceans (Niiyama 1959, Murofushi *et al*. 1984, Lécher *et al*. 1995). For example, for *P. clarkii* two reported diploid numbers has been dated for similar species with 2n=192 (Niiyama 1959) and 2n=188 (Murofushi *et al*. 1984), while in the Cambaridae *A. fluviatilis* 2n=116 (Lécher *et al*. 1995). Comparative chromosome data in other crustacean species is shown in Table 2. These studies suggest that the ranges of chromosome number in freshwater crayfish families (Astacidae, Cambaridae, and Parastacidae) are larger than that reported for palaemonoid prawn (Table 2). Such cytological settings represent a methodological problem for cytotaxonomist who wants to employ karyotypical characters as taxonomic key for species identification.

Karyotypical studies are still absent for several Mexican species, but comparative data are now available based on the present report for the tropical crayfish *P. llamasi* and the *P. diugeti* studied by Diupotex-Chong *et al*. (1997). The reported fundamental number (FN)

TABLE 2
Comparison of diploid chromosome (2n) number among three families of crayfish

Family	Species	2n	Source
Astacidae	*Astacus fluviatis*	116	Lécher *et al*. (1995)
Cambaridae	*Procambarus clarkii*	192	Niiyama (1959)
	P. clarkii	188	Murofushi *et al*. (1984)
	P. digueti	102	Diupotex-Chong *et al*. (1997)
	P. (A) llamasi	120	Current study
Parastacidae	*Cherax quadricarinatus*	200	Tan *et al*. (2004)

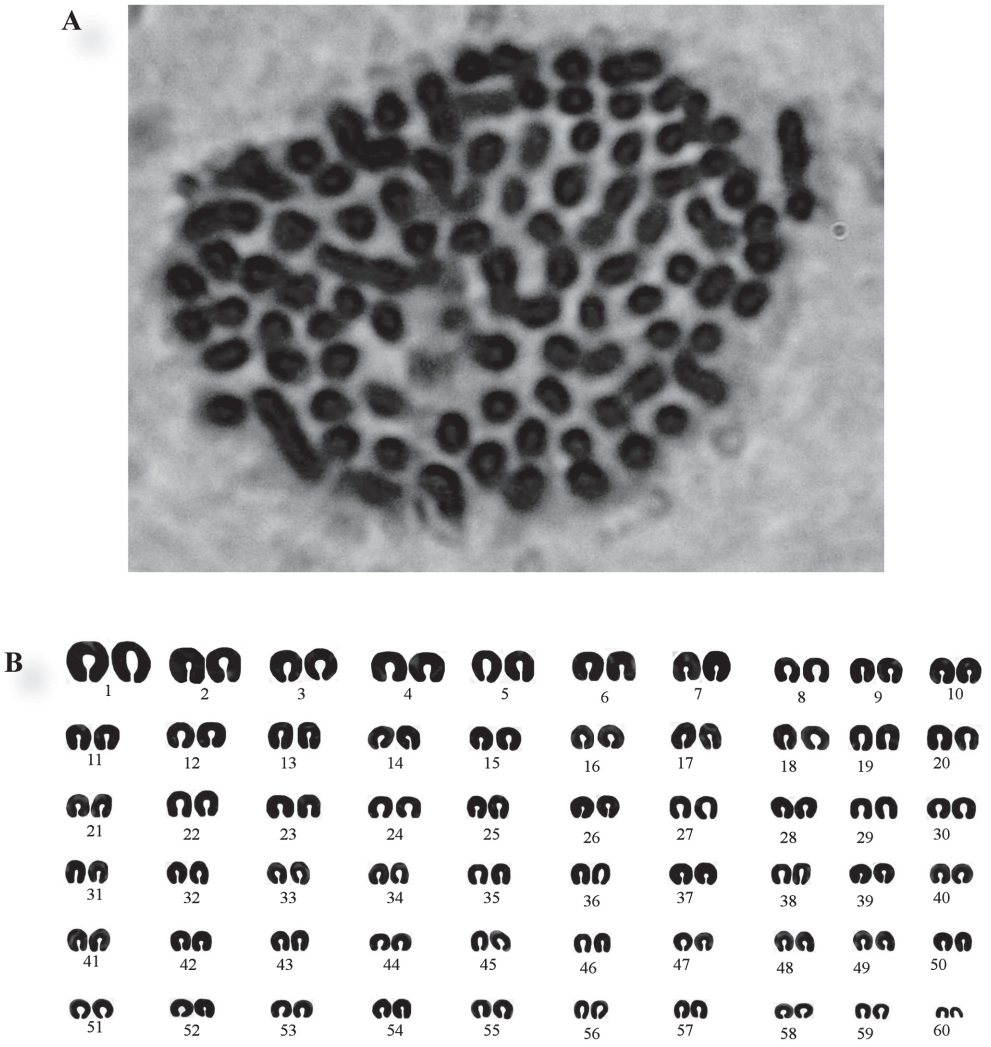

Fig. 2. Well-spread metaphase chromosomes of *P. llamasi* (A) and representative karyotype of *P. llamasi* with 2n=120 telocentric chromosomes (B).

of 204 autosomal arms for *P. diugeti* (Diupotex-Chong *et al*.1997), suggests that described karyotype structure of *P. llamasi* with NF of 120 arms is a cytotaxonomical key to separate both species, because their chromosome constitutions are quite different in the compared species. In addition both studies revealed that the presence of sex chromosomes is not a cytological characteristic of crayfish karyotype. Studies regarding chromosomes number, morphological taxonomy and molecular tools are still needed in many Mexican crayfish species to understand the evolutionary processes that promote their radiation in Mexico.

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RESUMEN

El género *Procambarus* ha recibido mayor atención en los estudios de los principios fundamentales de su biología debido a su diversidad en el territorio mexicano y potencial uso en acuicultura. El cariotipo típico del acocil tropical mexicano *Procambarus* (*Austrocambarus*) *llamasi,* se estudió mediante 189 dispersiones cromosómicas en metafase del tejido branquial de 17 adultos tratados con la técnica citológica de inmersión. Encontramos un amplio número de cromosomas, que variaron entre 98-120 elementos cromosómicos, con número modal diploide de 2n=120 elementos cromosómicos. El cariotipo del acocil tropical esta constituido por 60 pares de cromosomas monorrámeos, todos los centrómero están en la región telocéntrica de los cromosomas. En las metafases mitóticas de hembras y machos no fueron identificados cromosomas sexuales. Sugerimos considerar la estructura cromosómica del cariotipo como una herramienta citotaxonómica así como el empleo de datos cariológicos para propósitos de acuicultura y conservación del acocil tropical.

Palabras clave: cromosoma, cariotipo, acocil tropical, *Procambarus* (*Austrocambarus*) *llamasi.*

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