Reproduction of the tadpole shrimp *Triops* (Notostraca) in Mexican waters

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The gonadic histology of the tadpole shrimp *Triops* sp. from 22 Mexican locations was determined using light microscopy to investigate the hermaphroditic tissue. The sex ratio and type of morphotypes were also determined. Short morphotype individuals with ovisacs (58) were hermaphrodites and were recorded from 14 locations. Of the 34 long morphotype individuals with ovisacs, 25 were hermaphrodites recorded from seven locations, whereas nine were females recorded from only three locations. The proportion of males in hermaphrodite populations ranged from zero to 21%. Thus we infer that such populations have an androdioic reproduction. In female populations, it ranged from 50 to 62%; therefore such populations have a gonochoric reproduction.

Keywords: Androdioecy, gonadic histology, hermaphrodite, tadpole shrimp.

Introduction

THE crustaceans known as tadpole shrimp are grouped in the order Notostraca into a single family (Triopsidae) with two genera, *Triops* Schrank and *Lepidurus* Leach. These primitive genera are considered living fossils, as they have undergone minimum morphological changes over 150 million years[1,2]. From their adult external morphology, two types of individuals can be easily recognized: ovisac-bearing individuals (females or hermaphrodites), i.e. individuals that produce cysts (encysted embryos) having an ovisac (brood-pouch) in each thoracopod of the 11th body ring, and individuals without ovisac (males). The recorded proportion of these types of individuals in natural populations ranges from the nil to the dominance of males[3–6].

Isolated, ovisac-bearing individuals from populations without males are able to reproduce and so are considered to be hermaphrodites or parthenogenetic[7–9]. Bernard[7,10] was the first to report the presence of spermatic cells within the ovarian tissue in ovisac-bearing individuals of three species of tadpole shrimp (*Lepidurus glacialis*, *L. productus* and *Triops cancriformis*) and merely confirmed the existence of a hermaphroditic gonadic anatomy in these species. Since then, different morphological species have been reported with gonochoric or hermaphroditic reproduction, or both in the same species[9,11–13]. However, Trentini and Scanabissi[14] studied populations of *T. cancriformis* from Italy and found that the sex ratio was heavily biased in favour of ovisac-bearing individuals with a rudimentary form of hermaphroditism. They concluded that these populations had a parthenogenetic reproduction.

On the basis of the pedigree and sex ratio of North American *Triops* populations, Sassaman[15], and Sassaman *et al.*[5] proposed that populations composed mostly of ovisac-bearing individuals have an androdioecious reproduction. According to these authors, the androdioecy involves the participation of few males (genotype at the sex-determining locus = s/s), amphigenic hermaphrodites (S/s), and monogenic hermaphrodites (S/S). The amphigenic hermaphrodites produce offspring either by crossing with a male or by selfing. Thus the expected offspring sex ratios are 1 male : 1 amphigenic hermaphrodites when mated with a male, and 1 male : 2 amphigenic hermaphrodites : 1 monogenic hermaphrodite when selfed. The monogenic hermaphrodites produce offspring either by crossing with a male or by selfing. Thus, the expected offspring sex ratios are 100% amphigenic hermaphrodites when mated with a male and 100% monogenic hermaphrodites when selfed[15–18]. Longhurst[2], and Zaffagnini and Trentini[19] reported that *T. cancriformis* and *L. apus* showed a sex ratio that varied according to latitude. In northern Europe males are absent or rare; in central Europe the males when present are less in number than the females; in southern and western Europe and in North Africa the sex ratio is about 1 : 1.

Linder[4] proposed that all *Triops* species described from the American continent (including México) were synonyms of only one species, *T. longicaudatus*. However, Sassaman[5] demonstrated by allozyme differentiation and morphological analyses on populations from USA, that the nominal species *T. longicaudatus* is a mixture of at least two reproductively isolated species. Also, trypsin-

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like enzymes were found to differ between two uniparental forms from the Baja California Peninsula[20] and molecular analyses (12S rRNA mt DNA) showed that *Triops* populations from the same peninsula could represent several undescribed species[21]. Due to the diversity of notostracan species in North America, including México, a systematic re-evaluation of the populations using advanced genetic methods is needed. We prefer to keep all the Mexican forms of this genus under *Triops* sp. in this work.

In México, the tadpole shrimp *Triops* sp. has been reported mainly from arid and semiarid regions in 17 states of north and central México[22,23]. A study on the reproduction of Mexican *Triops* from two locations had reported two morphotypes with uniparental reproduction, presumably hermaphrodites[24]. Our objective is to contribute to the knowledge of the reproduction of Mexican *Triops*. Therefore, we focused on the following questions: Do hermaphrodites occur within the Mexican *Triops* populations? If so, what is the sex ratio in those populations? Are the hermaphrodites related to a specific morphotype? Do the hermaphroditic populations exhibit a geographic distribution pattern?

Methods

The *Triops* sp. populations studied are geographically distributed in eight states of México (Figure 1) and in two of the largest deserts (Chihuahuan and Sonoran) of North America. Shrimps were obtained directly from their natural habitats or from cultures. Cultures were made in 1200 l fibreglass tanks using commercial drinking water (total dissolved solids < 0.3 gl^{-1}) and 20 kg of dry soil containing *Triops* cysts as substrate. The presence of hermaphrodites was studied through gonadic histology and established by the positive record of hermaphroditic tissue (ovariotestes). The gonadic histology was made on adult ovisac-bearing individuals. Some adult males were also studied in order to have a reference of the testicular tissue. To obtain the histological preparations we followed the standard procedures described in Humason[25], and Howard and Smith[26]. The samples (shrimp) were washed with freshwater and fixed for 48 h in Davidson solution. Subsequently, they were dehydrated in a series of ethanol solutions (70%–I, 70%–II, 80%, 90%, 96%, 100%–I, and 100%–II), for 1 h in each solution. Then the samples were put in a solution of ethanol and xylene (1 : 1) for 15 min, and finally cleared with absolute xylene for 5–10 min. The samples were then put in a solution of paraffin and xylene (1 : 1) for 25 min and infiltrated in three paraffin baths in an oven at 60°C for about 1–2 h in each case. Histological slides of 3–6 μm thick were obtained with a rotating microtome (Leica RM2125), and mounted on glass slides. After removing the paraffin each slide was stained for 10 min with Harris' haematoxylin and counterstained for 6 min with eosin–phloxine. Finally, the slides were covered with synthetic resin and cover slips[27]. Gonadic sections were examined using a light microscope (Olympus BX41). The images were captured using a digital camera (cool SNAP-Pro color MediaCybernetics, San Diego, California, USA) and processed with the Image-Pro Plus software (version 5.0, MediaCybernetics, San Diego, California, USA). Sections with ovariotestes were further corroborated using the Fulgen method[26]. The slides were submerged in distilled water for 2 min, and hydrolysed in 5 N HCL at room temperature for 30 min. They were then washed with distilled water for 1 min, stained with Schiff's reagent for 15 min, and washed in distilled water. The slides were then stained with 0.03% picromethyl blue for 3 min and dehydrated in absolute ethanol–butanol. Finally the slides were dehydrated in three solutions of 100% butanol for 1–3 min, cleaned with xylene, and covered with synthetic resin and cover slips.

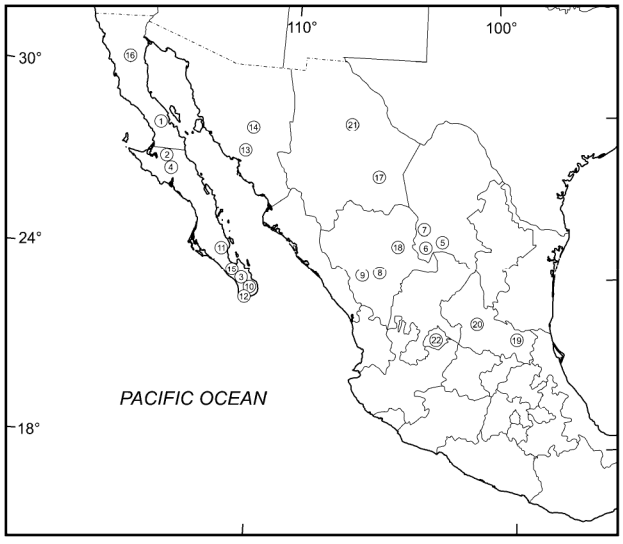

Figure 1. Geographical distribution of the Mexican *Triops* sp. populations studied. Numbers in circles correspond to the list of populations (locations) in Table 1.

The sex ratio and type of morphotypes present in the populations were determined by studying specimens collected from the field or from cultures. These specimens are available in crustacean collections of the Mexican institutions Centro de Investigaciones Biológicas del Noroeste, S.C. (CIB), Escuela Superior de Biología, Universidad Juárez del Estado de Durango (UJED), and Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León (FCB). Specimens with six or less body rings and without legs were determined as a short morphotype, and those with seven or more as a long morphotype.

Results

A total of 92 ovisac-bearing individuals and eight males of 22 populations (locations) were studied (Table 1). Ovisac-bearing individuals from 19 populations were anatomically hermaphrodites, i.e. testicular lobes were present within the tissue of the ovary (Figures 2 and 3).

Table 1. Locations, number of individuals studied (ovisac-bearing individuals and males), morphotype, number of histological slides and sections examined, number of ovisac-bearing individuals with ovariotestes, and type of reproduction. HE, Stained with haematoxilin and eosin

Location no.	Location	Ovisac-bearing individuals	Males	Morphotype	Number of histological slides		Number of histological sections		Ovisac-bearing with individuals ovariotestes	Type of reproduction
					HE	Fulgen	HE	Fulgen		
1	Km 39 to Bahía de Los Ángeles, Baja California	3	0	Large	25	4	68	12	3	Hermaphrodite
2	8.9 km S El Arco, Baja California Sur	5	0	Large	53	0	124	0	5	Hermaphrodite
3	Km 76.5 to Cabo San Lucas, Baja California Sur	3	0	Large	18	2	31	3	3	Hermaphrodite
4	500 mW Vizcaíno, Baja California Sur	4	0	Large	24	9	95	34	4	Hermaphrodite
5	El Refugio, Cerro Bola, 70 km E Torreón, Coahuila	3	0	Large	27	4	60	3	3	Hermaphrodite
		4	0	Short	24	11	70	28	4	Hermaphrodite
6	Saucillo, Viesca, Coahuila	4	1	Large	31	3	121	12	4	Hermaphrodite
7	Parque Béisbol Infantil, Torreón, Coahuila	3	1	Large	27	7	73	24	3	Hermaphrodite
		1	1	Short	17	3	35	6	1	Hermaphrodite
8	Arturo Bernal-Rancho Viborillas, Durango	1	1	Large	18	1	54	3	0	Gonochoric
9	Km 268 Zacatecas-Durango, Durango	5	1	Large	56	12	182	43	0	Gonochoric
10	Km 64 Cabo San Lucas-La Paz, Baja California Sur	5	0	Short	20	16	55	35	5	Hermaphrodite
11	Km 5 to San Juan de la Costa, Baja California Sur	5	0	Short	39	3	160	9	5	Hermaphrodite
12	Rancho Balmaceda, Baja California Sur	5	0	Short	36	15	183	44	5	Hermaphrodite
13	Km 89 Empalme-Cd. Obregón, Sonora	4	1	Short	36	0	112	0	4	Hermaphrodite
14	Junction Rancho El Coruco, Sonora	5	1	Short	84	0	292	0	5	Hermaphrodite
15	Laguna Seca, Baja California Sur	5	0	Short	23	6	70	16	5	Hermaphrodite
16	Km 79.3 Ensenada-San Felipe, Baja California	5	0	Short	52	5	152	15	5	Hermaphrodite
17	Estación Rellano-Los Sauces, Chihuahua	3	0	Short	30	6	96	25	3	Hermaphrodite
18	León Guzmán, Durango	5	0	Short	42	7	117	21	5	Hermaphrodite
19	Santa Gertrudis, Ciudad del Maíz, San Luís Potosí	4	0	Short	30	14	65	39	4	Hermaphrodite
20	Km 152 to Matehuala, San Luís Potosí	5	0	Short	27	23	137	95	5	Hermaphrodite
21	Km 208 Zaragoza-Buenaventura, Chihuahua	2	0	Short	18	3	66	11	2	Hermaphrodite
22	Salto del Burro, Aguascalientes	3	1	Large	30	0	103	0	0	Gonochoric

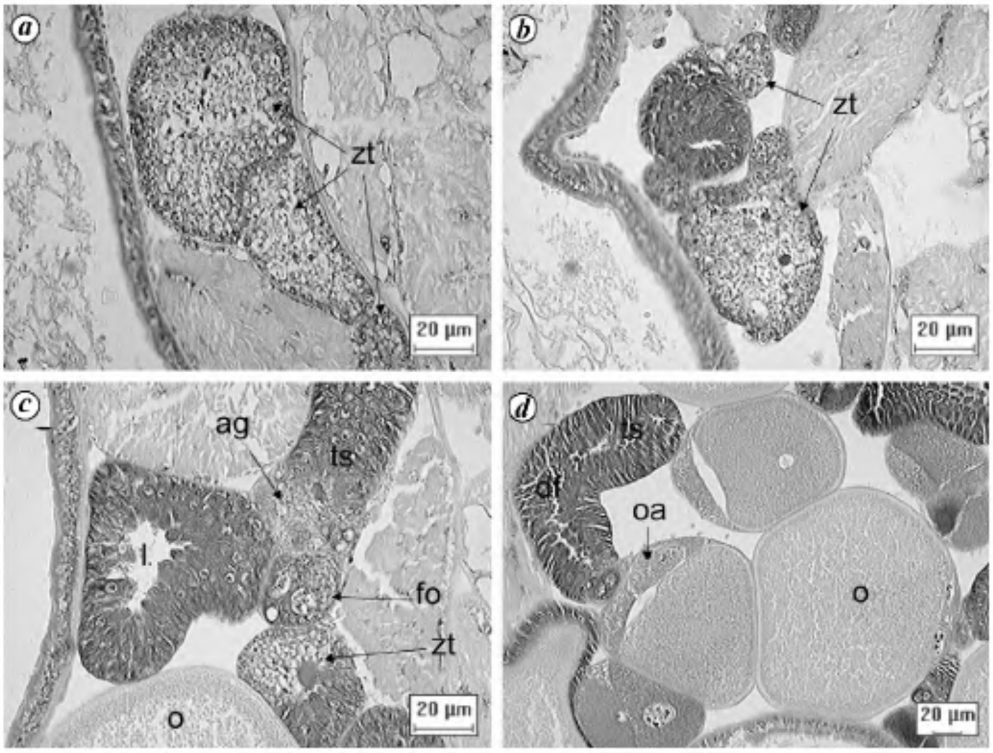

Figure. 2. Gonadic histological sections (stained with haematoxylin–eosin) of *Triops* sp. from 8.9 km S of El Arco, Baja California Sur, México. ***a–d***, Sections of a long morphotype hermaphrodite with ovariotestes. ***a***, ***b***, Somatic tubules with testicular zones. ***c***, Somatic tubules with a germinal area, ovarian follicle and testicular zone. ***d***, Somatic tubules with abortive oocytes and oocytes. ag, Germinal area; df, Follicular duct; fo, Ovarian follicle; l, Lumen; o, Oocyte; oa, Abortive oocyte; ts, Somatic tubule, and zt, Testicular zone.

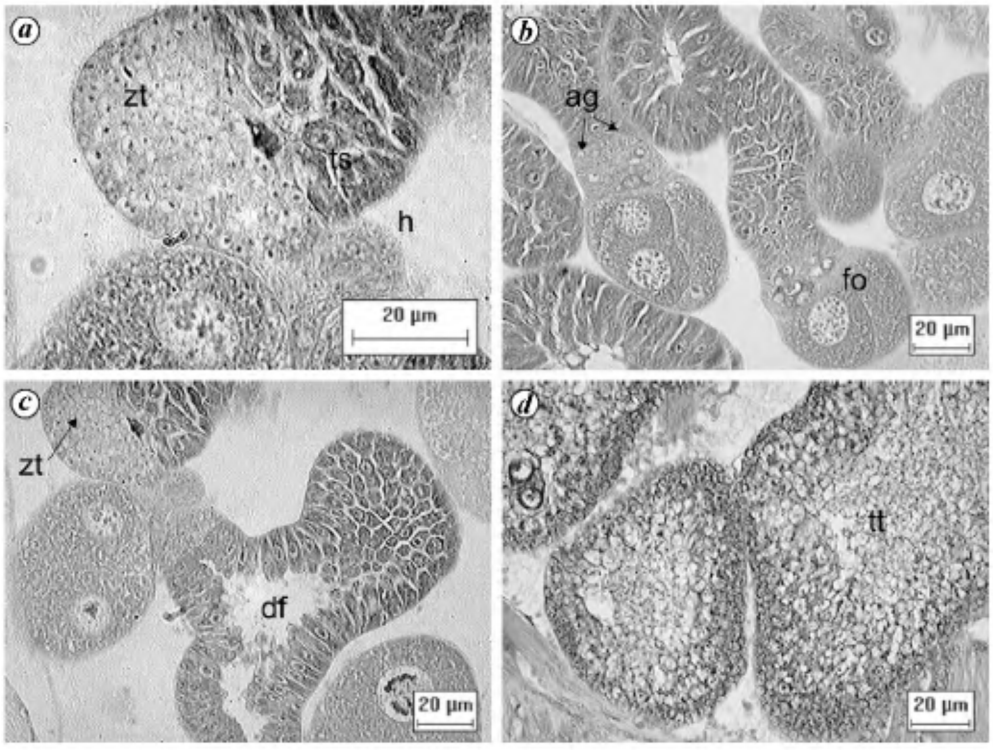

Figure 3. Gonadic histological sections (stained with haematoxylin–eosin) of *Triops* sp. from km 89, Empalme-Ciudad Obregón, Sonora, México. ***a–c***, Sections of a short morphotype hermaphrodite with ovariotestes. ***a***, Somatic tubules with a testicular zone. ***b***, Somatic tubules with germinal areas and ovarian follicles. ***c***, Somatic tubules with follicle duct and testicular zone. ***d***, Testicle section of a short morphotype male showing typical structure with testicular tubules. h, Haemocoel; tt, Testicular tubule.

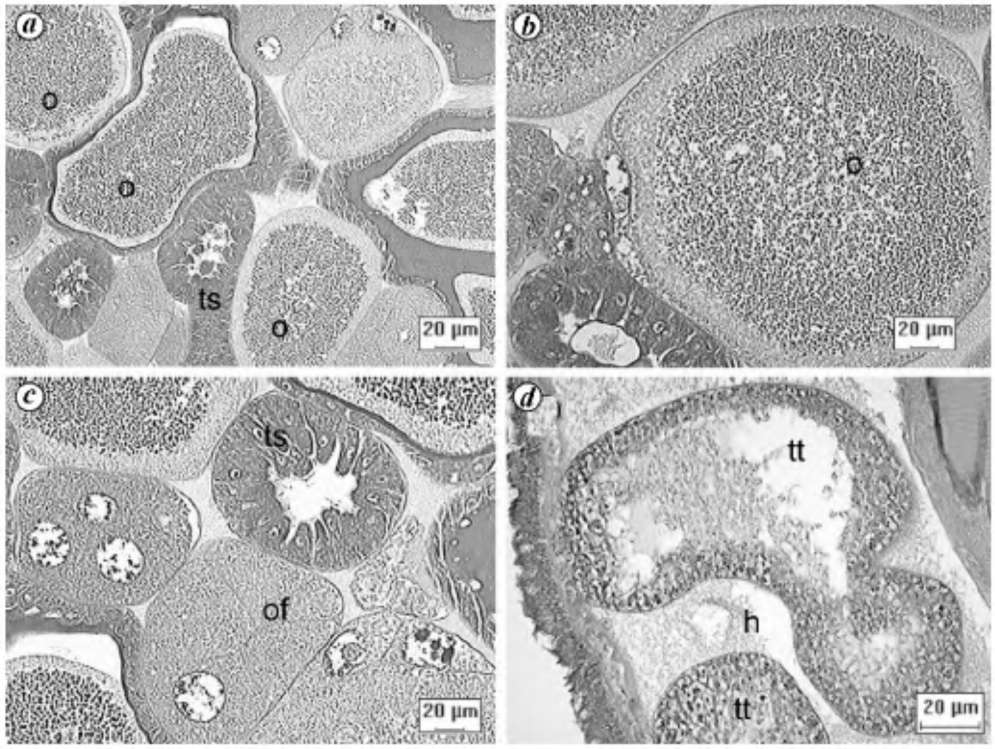

Figure 4. Gonadic histological sections (stained with haematoxylin–eosin) of *Triops* sp. from Arturo Bernal-Rancho Viborillas, Durango, México. ***a–c***, Ovarian sections of a long morphotype female showing typical structure with somatic tubules, oocyte follicles, and oocytes. ***d***, Testicle section of a long morphotype male showing typical structure with testicular tubules. of, Oocyte follicle.

No evidence of testicular lobes was found in ovisac-bearing individuals (Figure 4) from three populations (Table 1). These are found in the north-central part of the country in the southern region of the Chihuahuan desert. The rest of the populations with hermaphrodites are widely distributed in both the deserts (Chihuahuan and Sonoran).

Histological characteristics of gonads of the studied hermaphrodites, females, and males were typical, as described in previous studies[9,13,14,19,28,29]. Ovisac-bearing individuals had a pair of longitudinal gonads parallel to the midgut in the haemocoel. Multibranched tubules of somatic and germ cells were observed (Figures 3 and 4). In the hermaphrodites somatic tubules distributed from the second body ring up to the abdominal region were observed. Somatic tubules with ovariotestes (Figures 2 and 3) were mostly found in the thoracic region. The gonads in males were a pair of longitudinal testes located along the haemocoel on either side of the gut. We observed testicular tubules (Figures 3 and 4) in both the thoracic and abdominal regions.

In most of the studied populations, males were not recorded (Table 2). The sex ratio was close to 1 : 1 in only three sites; these corresponded to the original sites of ovisac-bearing individuals with no evidence of testicular lobes.

Discussion

Previous studies on the gonadic anatomy and sex ratio of the American populations of *Triops* revealed the occurrence of hermaphrodites and gonochoric individuals. Thus, ovariotestes had been found in ovisac-bearing individuals from California, USA[1,12,29]. Grigarick *et al.*[11] reported that *T. longicaudatus* from the rice fields of California, USA exhibited both gonochoric and hermaphrodite reproduction. Our present work is the first gonadic histological study covering a large geographical area in North America. In 86% of the studied populations, the ovariotestes were present in ovisac-bearing individuals. The proportion of males in these populations ranged from zero to 21% (Table 2). Thus we infer that such populations have a androdioic mode of reproduction. Sassaman[15], and Sassaman *et al.*[5] were the first to propose that some *Triops* populations from USA, with a highly varied proportion of males (0–0.4), have a androdioic mode of reproduction. In the remaining Mexican populations the ovisac-bearing individuals exhibited no evidences of ovariotestes and the proportion of males ranged from 50 to 62%. Therefore, these populations have a gonochoric reproduction. These hermaphrodite and gonochoric populations are being studied for their genetic and morphological identity (mt DNA) and preliminary results suggest that there is a separation between them at the species level[21] (H. Obregón-Barboza, unpublished). Longhurst[1], in his systematic review defined that *T. cancriformis*, *T. longicaudatus*, *L. apus* and *L. articus* show both gonochoric and hermaphrodite reproduction, whereas *T. aus traliensis*, *T. granarius*, *L. batesoni* and *L. lemmoni* (cited as *L. lynchi*) have a gonochoric reproduction (cited as bi-

Table 2. Sex ratio recorded in Mexican *Triops* populations

Location no.	Location	Source	Collection date	Catalogue number	Males	Ovisac-bearing individuals	Proportion of males (%)
1	Km 39 to Bahía de Los Ángeles, Baja California	Field	21.06.1995	CIB-065	0	18	0
2	8.9 km S El Arco, Baja California Sur	Culture	16.06.2000	CIB-514	0	1	0
3	Km 76.5 to Cabo San Lucas, Baja California Sur	Field	07.10.1995	CIB-133	0	1	0
		Field	22.10.1995	CIB-124	0	7	0
4	500 m W Vizcaíno, Baja California Sur	Field	29.08.1996	CIB-259	0	26	0
5	El Refugio, Cerro Bola, 70 km E Torreón, Coahuila	Field	25.11.1984	UANL-131	0	33	0
		Field	25.11.1984	UJED-098	0	5	0
6	Saucillo, Viesca, Coahuila	Field	15.05.1981	FCB-163	0	3	0
		Field	05.05.1981	UANL-135	1	22	4
		Field	15.05.1981	UANL-139	0	17	0
		Culture	04.06.2001	CIB-577	3	29	9
7	Parque Béisbol Infantil, Torreón, Coahuila	Field	10.07.1985	UJED-115	3	15	17
		Culture	23.08.2001	CIB-592	2	13	13
		Field	10.07.1985	UJED-113	0	15	0
8	Arturo Bernal-Rancho Viborillas, Durango	Field	16.07.1985	FCB-205	23	14	62
		Culture	24.08.2001	CIB-605	1	1	50
		Culture	31.08.2001	CIB-607	3	3	50
9	Km 268 Zacatecas-Durango, Durango	Field	16.07.1985	FCB-203	1	1	50
		Field	16.07.1985	UJED-103	6	6	50
		Field	20.10.2002	CIB-610	57	51	53
		Culture	07.05.2003	CIB-690	10	11	48
10	Km 64 Cabo San Lucas-La Paz, Baja California Sur	Culture	13.12.1999	CIB-471	0	17	0
11	Km 5 to San Juan de la Costa, Baja California Sur	Field	29.09.1996	CIB-325	0	1	0
		Field	09.10.2001	CIB-636	0	37	0
12	Rancho Balmaceda, Baja California Sur	Culture	11.09.2000	CIB-526	0	7	0
13	Km 89 Empalme-Cd. Obregón, Sonora	Field	07.09.1996	CIB-298	3	36	8
		Culture	25.05.2001	CIB-617	9	104	8
		Culture	03.06.2002	CIB-670	8	73	10
		Culture	27.09.2002	CIB-672	4	15	21
14	Junction Rancho El Coruco, Sonora	Field	08.09.1996	CIB-306	0	7	0
		Culture	14.05.2001	CIB-622	11	236	4
15	Laguna Seca, Baja California Sur	Culture	27.09.2002	CIB-637	0	22	0
16	Km 79.3 Ensenada-San Felipe, Baja California	Field	28.09.1997	CIB-415	5	29	15
17	Estación Rellano-Los Sauces, Chihuahua	Field	16.07.1983	UJED-125	0	2	0
18	León Guzmán, Durango	Field	10.09.1983	UJED-117	0	2	0
19	Santa Gertrudis, Ciudad del Maíz, San Luis Potosí	Field	30.07.1999	CIB-443	0	5	0
20	Km 152 to Matehuala, San Luís Potosí	Field	29.07.1999	CIB-441	0	105	0
21	Km 208 Zaragoza-Buenaventura, Chihuahua	Culture	12.06.2003	CIB-657	0	8	0
22	Salto del Burro, Aguascalientes	Field	23.06.1999	CIB-627	3	3	50
		Field	27.06.1999	CIB-628	3	3	50

sexual). Zaffagnini and Rossi[28] studied ovisac-bearing individuals of an Italian *T. cancriformis* population without males and reported that the ovariotestes were present in all of them. However, the hermaphroditic reproduction is still inconclusive given the absence of direct cytogenetic evidence on the mechanism of restoration of diploidy by the haploid oocytes[16].

The self-fertilization of hermaphrodites is considered as degenerated sexuality. According to Williams[30], the hermaphroditic populations tend towards a complete individual homozygosity, but frequently with high levels of polymorphism in the population. Their existence demonstrates that these genomes have evolved tolerating high levels of homozygosity. The incidence of a low percentage of males in Mexican *Triops* hermaphroditic populations suggests that their reproduction is not strictly uniparental, but a androdioic one. This condition probably has a dual function, i.e. whereas an autocompatible hermaphrodite (amphigenic or monogenic) functions as an ideal colonizer (a population can be established from a single individual), the presence of males (originated from amphigenic hermaphrodites) could function as a mediator system of the effects produced by endogamy. The androdioecy has been well documented in branchiopods of temporary waters[15–18]. The success level of aptitude of the amphigenic hermaphrodites in clam shrimp of the genus *Eulimnadia* is ca. 13% better than the monogenic hermaphrodites, and thus secures the presence of males in the androdioic populations[31]. Hermaphroditic reproduction has been suggested to be derived from gonochoric reproduction with females

producing testicular lobes in the ovaries[18]. In the gonochoric species the females are the heterogametic sex, and this sex-determining condition has been retained in the androdioic species. Thus the males are determined by a recessive allele[18].

In the Old World, recent records of German biparental *Triops* populations, north of parallel 50° do not support the old concept that the modes of reproduction (hermaphroditic and gonochoric) are related to a north-south gradient[32]. In USA, gonochoric populations of *Triops* '*longicaudatus*' have been recorded from the western and central part of the country[5]. Results of this study indicate that there is no latitudinal distribution of the two types of Mexican *Triops* sp. populations. Hermaphrodite populations are widely distributed and the gonochoric populations were found in three locations in north-central México.

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