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On a new isolate of *Heterorhabditis bacteriophora* Poinar, 1975 (Nematoda : Heterorhabditidae) from Argentina : life cycle and description of infective juveniles, females, males and hermaphrodites of 2nd and 3rd generations

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Summary – A new isolate of *Heterorhabditis bacteriophora* Poinar, 1975 from Oliva, Argentina is described. The life cycle was studied under laboratory conditions. Three generations were observed : a first hermaphroditic generation, a second generation in which both hermaphroditic and amphimictic form occur, and a third generation, hermaphroditic. The morphological and morphometric characters were analysed. A comparative study of typical and Rio Cuarto isolates found morphological characters to be similar. Significant differences between Oliva and Río Cuarto isolates were observed for most of the measurements of adults of the first and second generations and infective juveniles. The hermaphrodites of the second and third generation, described for the first time, are differentiated by the length and shape of the tail and the shape of the vulva.

Résumé – ***A propos d'un nouvel isolat d'*Heterorhabditis bacteriophora *Poinar, 1975 (Nematoda : Heterorhabditidae) d'Argentine : cycle biologique et description des juvéniles infestants, des femelles, mâles et hermaphrodites de deuxième et troisième générations*** – Un nouvel isolat d'*Heterorhabditis bacteriophora* Poinar, 1975 est décrit. Le cycle biologique a été étudié dans les conditions du laboratoire. Trois générations ont été observées : une hermaphrodite, une dans laquelle les deux formes hermaphrodite et amphimictique apparaissent et une hermaphrodite. Une étude comparative de l'isolat typique et de l'isolat de Rio Cuarto a été faite, les caractères morphologiques étant similaires. Des différences significatives entre les isolats Oliva et Rio Cuarto ont été observées pour la plupart des mensurations des adultes de la première et de la deuxième générations et pour les juvéniles infestants. Les hermaphrodites des deuxième et troisième générations, décrits pour la première fois, sont différenciés par la longueur et la forme de la queue et par la forme de la vulve.

Key-words : *Heterorhabditis bacteriophora,* life cycle, nematode.

Heterorhabditis bacteriophora was first found in Brecon, Australia (Poinar, 1975 *a*). Since then, more than twenty other isolates from different regions of the world have been mentioned; each of them differs in behaviour and physiology (Poinar, 1990).

Samplings, conducted in the province of Córdoba, revealed the presence of this nematode in Oliva (OLI isolate) and Río Cuarto (RIV isolate) (Doucet, 1990). Laboratory observations showed that OLI possesses a greater infective capacity than RIV and that the coloring found in the parasitized insects is different (violaceous-brown *vs* reddish respectively) (Doucet *et al.,* 1992).

Previous studies of the life cycle of this nematode indicated that it is heterogonic, its first generation being hermaphroditic (1HG) and the second one amphimictic (2AG) (Poinar, 1975 *a*), followed by other generations depending on the quantity and quality of nutrients (Poinar & Hansen, 1983). The sequence of generations was described in different ways by different authors : some pointed out a third hermaphroditic generations (3HG) (Poinar, 1975 *a;* Milstead & Poinar, 1978); others, one or more amphimictic generation (2AG; 3AG; ... nAG) always after the first hermaphroditic generation (1HG) (Poinar & Hansen, 1983; Poinar, 1990). Recent studies show an uninterrupted sequence of HG (Glazer *et al.,* 1991; Zioni *et al.,* 1992 *b*).

This work characterizes the isolate found in Oliva and its life cycle. For the first time the hermaphrodites of the second and third generation are described.

Material and methods

H. bacteriophora was detected in Oliva (OLI isolate) and Río Cuarto (RIV isolate) using the rapid method for extracting entomophagous nematodes (Doucet, 1986).

The nematodes were reared on *Galleria mellonella* (L.) (Lepidoptera : Pyralidae) larvae (0.20-0.25 g); the intensity was from one to ten infective juveniles (IJ) insect, and the temperature between 28-29 °C (Doucet *et al.,* 1991). Observations of the life cycle and extraction of the adult stages were carried out by daily dissections of five parasitized hosts.

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The isolates of Brecon (Poinar, 1975 *a*), RIV (Doucet & Doucet, 1986) and OLI were compared. In order to evaluate the differences between the RIV and OLI isolates, the Mann-Whitney U test and ANOVA were applied (Sokal & Rohlf, 1980).

Morphological and morphometric studies were carried in specimens mounted on permanent preparations (Doucet, 1980); hermaphrodites and females in older specimens were definined by the ovotestis position (Stanuszek, 1974).

The characters taken into account were selected from among those usually used in the descriptions of *H. bacteriophora* (Poinar, 1975 *a, b;* Doucet & Doucet, 1986). For each character, the range, mean, standard deviation and coefficient of variation were calculated. Hermaphrodites 2HG and 3HG were compared by ANOVA and Mann-Whitney U test (Sokal & Rohlf, 1980).

Variability was considered low when the CV values were below 10 %; intermediate between 10 and 20 %, and high above 20 % (Stanuszek, 1974).

Results

LIFE CYCLE (Fig. 1)

The only stage found outside the insect was IJ, which represents the free phase and carries out the dispersion and attacks on a new host.

The nematode killed the insect 24 to 48 h after contact; the coloring of the host body changed from the typical grey to violaceous-brown.

The parasitic period lasted 15 days from the moment IJ entered the insect to the time when new IJs were formed. During this phase, there was a succession of three generations : the first one with hermaphrodites (1 HG) (2-5 days after the death of the insect); the second one with females and males (2 AG) (4-9 days) and hermaphrodites (2 HG) (9-12 days); the third one with hermaphrodites (3 HG) (12-15 days). As a result of the development of eggs (E), the four typical stages of nematode juveniles (J1, J2, J3, J4) were found in all cases, and also the IJ characteristic of Heterorhabditidae. The J2 from 1 HG continued their development into two types of J3 : those which matured into J4 and those which originated the IJs. The formation of these two types of juveniles appeared to depend on the place where eggs developed : J3 were formed from the eggs released in the haemocoel and IJs from those which remained inside the body of hermaphrodites 1 HG. The J2 formed by 2 HG and 2 AG became 3 HG or IJs.

CHARACTERIZATION

Measurements

(See Tables 1, 2 and 3)

Description

Hermaphrodites (1 HG), females and males (2 AG) and IJs : Morphological characters are similar to those of the type and RIV isolates (Poinar, 1975*a;* Doucet & Doucet, 1986).

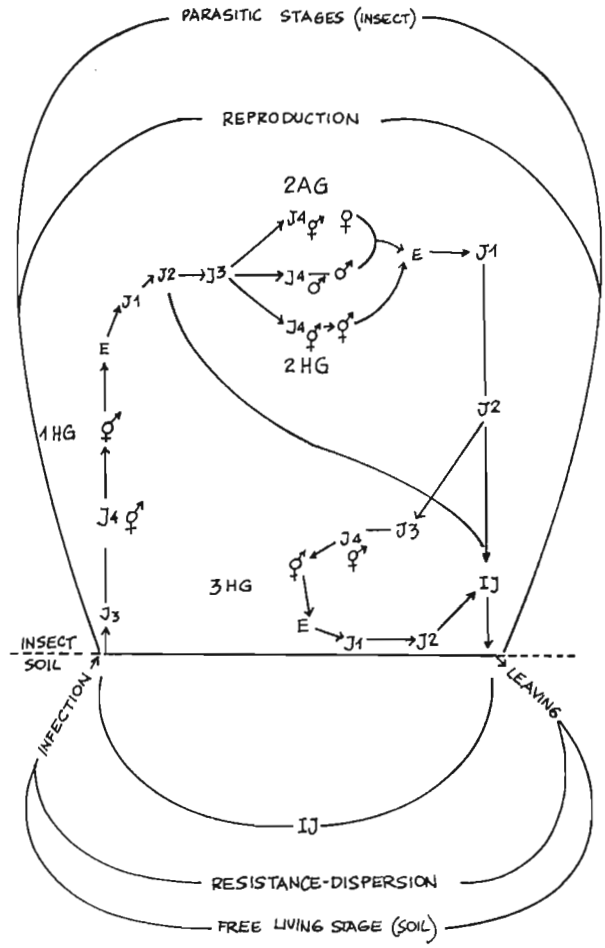

Fig. 1. *Life cycle of* Heterorhabditis bacteriophora *OLI. (E = egg; J1 to J4 = juveniles; IJ = infective juvenile; HG = hermaphroditic generation; AG = amphimictic generation).*

Hermaphrodites (2 HG) (Fig. 2) : Thin and elongate body; circular section and " habitus " slightly curved. Anterior end rounded. Terminal oral opening surrounded by six labial papillae. Buccal cavity wide, with strongly cuticularized walls. Œsophagus elongate, clavate; basal bulb valvate and piriform; isthmus surrounded by nervous ring. Excretory pore ventrally located close to level of base of œsophagus. Vulva located at midbody, with prominent vulvar lips. Reproductive system amphidelphic and reflexed; ovotestis located at midbody; oviduct and uterus with thick walls; presence of eggs in various stages of development; vagina short with muscularized walls. Tail thin, elongate (length three times body width at anus level) and pointed. Region posterior to anus bulky.

Table 1. *Measurements of hermaphrodites and females of* Heterorhabditis bacteriophora *Poinar 1975, from Oliva (OLI) and Río Cuarto (RIV) Córdoba, Argentina, and from Brecon (type isolate), Australia.*

Characters	Hermaphrodites(1)			Females		
	Córdoba isolate		Type isolate	Córdoba isolate		Type isolate
	OLI (n=20)	RIV (n=20)	(n=15)	OLI (n=20)	RIV (n=20)	(n=15)
Body length (mm)	4.8 ± 0.55 (3.9-5.8) 11.44	5.09 ± 0.38* (4.2-5.6) 13.28	4.03 (3.63-4.39)	2.22 ± 0.21 (1.8-2.6) 9.33	2.11 ± 0.19 (1.8-2.4) 9.06	3.50 (3.18-3.85)
Body diameter	215.7 ± 27.64 (177.5-255) 12.81	204.1 ± 15.2 (180-242) 7.44	165 (160-180)	114.13 ± 9.81 (97.5-133) 8.6	121.15 ± 14.38 (100-162) 11.87	190 (160-220)
Length of stoma	11.13 ± 1.9 (7.5-15) 17	14.5 ± 1.05** (13-16) 7.25	8 (6-9)	7.88 ± 1.47 (5-10) 18.63	10 ± 0.45** (9-11) 4.5	7 (6-9)
Width of stoma	11.13 ± 1.89 (7.5-12.5) 13.28	10.6 ± 0.82 (10-12) 7.74	8 (6-9)	8.25 ± 1.43 (5-10) 17.31	9.05 ± 0.6 * (8-10) 6.68	7 (6-9)
Œsophagus length	183.7 ± 14.06 (162.5-207) 7.65	199.2 ± 6.77 ** (180-215) 3.39	197 (189-205)	137.25 ± 6.48 (125-148) 4.72	132.2 ± 7.2 * (108-140) 5.45	168 (155-183)
Tail length	81 ± 7.09 (70-95) 8.75	74.3 ± 5.78** (65-87) 7.78	90 (81-93)	71.5 ± 4.32 (62.5-80) 6.05	54.1 ± 5.51** (40-65) 10.19	82 (71-93)
Tail diameter	51.13 ± 5.82 (42.5-62.5) 11.38	53 ± 2.8 (50-57) 5.3	46 (40-53)	31.75 ± 3.15 (22.5-37.5) 9.93	27.3 ± 3.69** (23-40) 13.5	28 (22-31)
Distance between anterior end and excretory pore	194.38 ± 15.53 (175-225) 7.99	192.9 ± 12.9 (175-225) 6.7	209 (189-217)	165-7.4 (152.5-175) 4.48	149.3 ± 6.95** (138-162) 4.65	192 (174-214)
Distance between anterior end and nerve ring	77.88 ± 7.71 (67.5-92.5) 10	138.7 ± 7.42** (125-150) 5.35	16 (121-130)	65 ± 7.43 (55-87.5) 11.44	91.65 ± 4.27** (83-100) 4.65	103 (93-118)
V	41.83 ± 2.1 (36.1-45.1) 4.81	40.25 ± 1.55** (37-44) 3.85	44 (41-47)	49.88 ± 5.2 (40.7-69.7) 10.43	46.6 ± 2.58** (41-50) 5.54	47 (42-53)

*, ** Differences between OLI and RIV isolates significant at $P < 0,05$; $P < 0,01$ respectively (Mann - Whitney U test and Model 1 anova). All measurements are in μm, except Body length. The disposition of the measurements corresponds to the following arrangement : arithmetic mean ± standard deviation (range); coefficient of variation.
(1) Only first generation.

Hermaphrodites (3 HG) (Fig. 2) : Similar to those described above, they differ in having a thick body; slightly developed vulvar lips, conical tail robust and short (length twice the body width at anus level).

Comparison of OLI with Brecon and RIV isolates

The characteristics of the OLI isolate show slight differences with respect to isolates from Australia (Poinar, 1975*a*) and Río Cuarto (Córdoba) (Doucet & Doucet, 1986), mainly regarding morphometric characters. This widens the range of variation previously known (Poinar, 1990) for all stages of this species, except for maximum body width and ratios " a " and " d " in IJs; width of stoma and tail length in males; length of stoma and distance from anterior end of excretory pore in 1HG; and body width at anus level and distance from anterior end to excretory pore in females 2 AG (Tables 1, 2). Most of the morphometric characters for hermaphrodites (1 HG), females and males (2 AG), and IJs exhibited significant differences between OLI and RIV.

Variability of the morphometric characters

Morphometric characters differ according to the stage under consideration. The coefficient of variation (CV)

Table 2. *Measurements of males and infective juveniles of* Heterorhabditis bacteriophora *Poinar 1975 from Oliva (OLI) and Río Cuarto (RIV) Córdoba, Argentina, and from Brecon (type isolate), Australia.*

Characters	Males			Infective juveniles		
	Córdoba isolate		Type isolate	Córdoba isolate		Type isolate
	OLI (n=20)	RIV (n=20)	(n=15)	OLI (n=20)	RIV (n=20)	(n=15)
Body length (mm)	0.91 ± 0.06 (0.8-1.05) 6.46	0.78 ± 56.12** (0.72-0.94) 6.61	0.82 (0.78-0.96)	0.54 ± 0.03 (0.49-0.61) 6	0.59 ± 29.57** (0.54-0.64) 4.95	0.57 (0.52-0.60)
Body diameter	48.05 ± 3.86 (40-57) 8.03	44.5 ± 2.7** (37-47) 6.07	43 (38-46)	23 ± 1.03 (22-25) 4.46	25.45 ± 2.35** (22-29) 9.23	24 (21-31)
Length of stoma	7.15 ± 0.93 (6-9) 13.05	6 ± 0.6** (5-7) 9.36	3 (2-4)	–	–	–
Width of stoma	5.25 ± 0.55 (4-6) 10.49	5.05 ± 0.51 (4-6) 10.10	2 (1-3)	–	–	–
Œsophagus length	101.4 ± 4.69 (92.5-107.5) 4.63	102.3 ± 3.34** (95-110) 3.26	103 (99-105)	112 ± 4.68 (103-119) 4.18	121.4 ± 4.92** (110-130) 4.05	125 (119-130)
Tail length	28.9 ± 2.27 (24-33) 7.85	24.35 ± 1.56** (20-27) 6.42	28 (22-36)	89.6 ± 10.22 (72-105) 11.4	93.4 ± 4 (85-100) 4.28	91 (83-99)
Tail diameter	20 ± 1.03 (18-22) 5.13	22.2 ± 1.32** (20-25) 5.95	23 (22-25)	14.9 ± 1.8 (11-17) 12.1	15.65 ± 0.81 (15-17) 5.19	–
Distance between anterior end and excretory pore	125.9 ± 7.6 (113-140) 6.02	129.15 ± 4.57** (120-137) 3.61	121 (114-130)	93.95 ± 2.96 (87-101) 3.16	100.4 ± 3.74 (96-110) 3.73	104 (94-109)
Distance between anterior end and nerve ring	51.9 ± 4.45 (45-62) 8.57	77.25 ± 3.43** (70-85) 4.44	72 (65-81)	77.45 ± 2.65 (73-84) 3.42	84.2 ± 2.33 (80-88) 2.76	83 (81-88)
Distance between anterior end and reflexed portion of testis	96.2 ± 31.65 (54-210) 32.9	85.05 ± 8.06** (75-100) 9.48	–	–	–	–
Distance between reflexed portion of testis and anus (mm)	0.68 ± 0.05 (0.56-0.79) 7.6	0.62 ± 0.05** (0.52-0.7) 7.73	–	–	–	–
Length of spicules	46.8 ± 2.86 (40-51) 6.1	43.2 ± 1.96** (39-47) 4.54	40 (36-44)	–	–	–
Length of gubernaculum	22.9 ± 2.22 (19-27) 9.7	20.6 ± 1.35** (18-24) 6.57	20 (18-25)	–	–	–
Ratio a	–	–	– 4.3	23.44 ± 1.01 (20.4-25) 4.3	23.6 ± 1.5 (20-26) 6.44	–
Ratio b	–	–	–	4.77 ± 0.24 (4.3-5.2) 5	4.92 ± 0.30 (4.5-5.8) 6.12	–
Ratio c	–	–	–	6.07 ± 0.79 (4.9-7.6) 13.07	6.39 ± 0.19* (6.1-6.8) 3.02	–
Ratio d(1)	–	–	–	0.84 ± 0.02 (0.8-0.88) 1.97	0.82 ± 0.05 (0.75-0.9) 5.6	–
Ratio e(2)	–	–	–	1.00 ± 0.14 (0.8-1.3) 13.88	1.07 ± 0.06* (0.98-1.2) 5.47	– –

*, ** Differences between OLI and RIV significant at $P \leq 0.05$; $P \leq 0.01$ respectively (Mann-Whitney U test and Medel I anova). All measurements are in µm, except Body length and Distance beetwen reflexed portion of testis and anus. The disposition of the measurements corresponds to the following arrangement; arithmetic mean ± standard deviation (range); coefficient of variation.

(1) Distance between anterior end and excretory pore divided by esophagus length.

(2) Distance between anterior end and excretory pore divided by tail length.

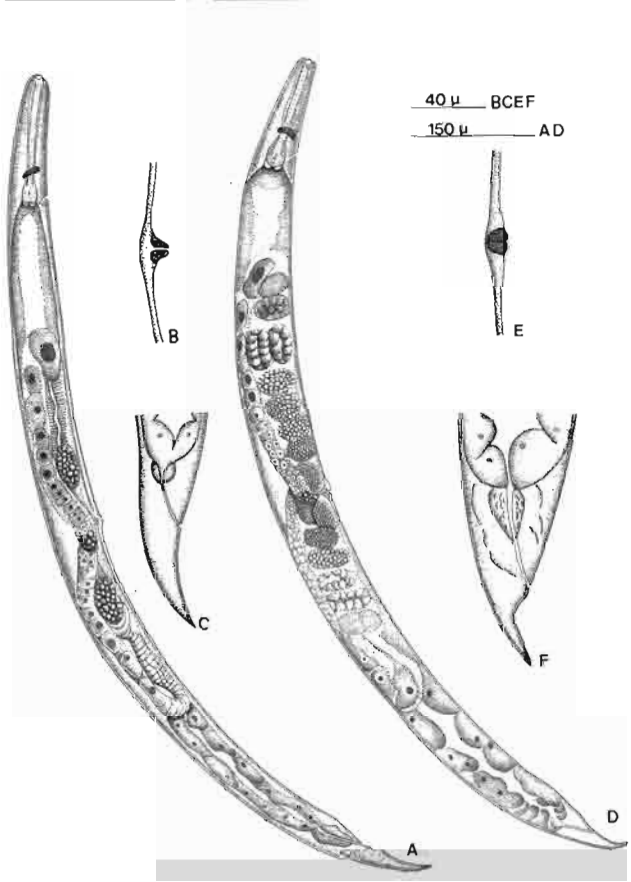

Fig. 2. Heterorhabditis bacteriophora *OLI. Hermaphroditic female of second generation. A : whole body; B : Vulval region (lateral); C : Posterior region (lateral) – Hermaphroditic female of third generation; D : whole body; E : Vulval region (lateral); F : Posterior region (lateral).*

is generally low in IJs and males (Table 2). Exceptions in IJs : tail length (CV : 11.4), body width at anus level (CV : 12.1), ratios c (CV : 13.07) and e (CV : 13.88); in males : length of testis from anterior end to flexion (CV : 32, 9). The variability in 1 HG, 2 HG, and 2 AG was intermediate. The same was found on 3 HG, except for the characters : tail length (CV : 22), ratios c (CV : 24.99) and e (CV : 22.47), which were highly variable (Table 3). Variability of ratio V was always low with values close to 5 %, except in 2 AG (CV : 10.43) (Tables 1, 3).

Both the Mann-Whitney U test and ANOVA showed significant differences between 2 HG and 3 HG ($P \leq 0.01$ or 0.05) in total body length, tail length, distance from anterior end to excretory pore, stoma width and ratios a, b, c, c', d, e (Table 3).

Discussion

Hermaphrodites of the second and third generations of *H. bacteriophora* are described for the first time.

Table 3. *Measurements of the hermaphrodites of second and third generation of* Heterorhabditis bacteriophora *OLI from Córdoba.*

Characters	Second generation (n=13)	Third generation (n=13)
Body length (mm)	1.05 ± 0.13 (0.82-1.22) 11.99	1.26 ± 0.17** (0.96-1.61) 13.94
Body diameter	68.07 ± 8.5 (50-87.5) 12.49	70.07 ± 6.76 (62-80) 9.65
Length of stoma	11 ± 1.08 (9-13) 9.78	10 ± 1.22 (7-11) 12.24
Width of stoma	7.13 ± 0.81 (6-9) 11.35	6.38 ± 0.76* (5-7) 12.03
Esophagus length	144.6 ± 13.6 (125-157.5) 9.38	140.53 ± 14.59 (117.5-165) 10.38
Tail length	60.23 ± 8.58 (50-80) 14.24	44.84 ± 9.88** (32.5-70) 22
Tail diameter	22.05 ± 1.9 (20-25) 8.6	21.5 ± 1.68 (19-25) 7.82
Distance between anterior end and excretory pore	135.6 ± 11 (120-146) 8.13	117.57 ± 9.5** (104-140) 8.08
Distance between anterior end and nerve ring	98.9 ± 10 (85-115) 10.16	94.65 ± 10.83 (78-112.5) 11.44
Ratio a	15.72 ± 1.73 (12.55-18.07) 11	18 ± 1.52** (15.23-20.12) 8.47
Ratio b	7.23 ± 0.72 (5.57-8.16) 10	9.07 ± 1.48** (6.76-11.16) 16.4
Ratio c	17.09 ± 2.66 (13.12-24.4) 15.61	29.35 ± 7.33** (19.13-40.28) 24.99
Ratio c'	2.9 ± 0.6 (2-4) 20.7	2.08 ± 0.38** (1.59-2.8) 18.46
Ratio d	0.92 ± 0.05 (0.88-1.04) 5.65	0.83 ± 0.08** (0.75-0.98) 10.16
Ratio e	2.19 ± 0.26 (1.73-2.7) 12.09	2.72 ± 0.6** (1.68-3.93) 22.47
V	49.39 ± 2 (46.72-53.8) 4.05	50.33 ± 2.6 (45.33-53.33) 5.16

*, ** Differences between OLI and RIV significant at $P \leq 0.05$; $P \leq 0.01$ respectively (Mann-Whitney U test and Model I anova). All measurements are in µm except Body length. The disposition of the measurements corresponds to the following arrangement; arithmetic mean ± standard deviation (range); coefficient of variation. Ratios d, e; see Table 2.

Morphologically, these hermaphrodites are similar to those of the first generation, but smaller. As new generations appear and the availability of nutrients diminishes, the individuals are smaller and smaller (Poinar, 1979; Zioni *et al.*, 1992a). However, contrary to what may be expected, hermaphrodites 3 HG are larger than the 2 HG hermaphrodites. It is probable that competition for nutrients between 2 AG and 2 HG is the cause of these differences (Begon *et al.*, 1988).

The variability that characterizes this species (Poinar, 1975*a;* Doucet & Doucet, 1986; Doucet *et al.*, 1991), as well as other entomophagous nematodes (Poinar, 1979; Doucet, unpubl.) is also evident in this isolate. The least variable characters are those of IJs and males while the most variable ones are those of 1 HG and 2 AG. Character variability of 2 HG and 3 HG is intermediate (CV < 20) except for tail length in 3 HG (CV = 22), the only character that is highly variable. The morphometric characters extended the ranges previously known for the species.

The life cycle observed in this work comprises three successive generations; 1 HG, 2 HG – 2 AG and 3 HG. The succession of HG is common in a liquid medium (Poinar & Hansen, 1983; Zioni *et al.*, 1992*b*). However, the coexistence of HG and AG in the second generation is reported for the first time.

In light of the sequence of generations mentioned above, a second analysis of the hypothesis concerning the formation of hermaphrodites with respect to the quality and quantity of nutrients (Paramonov, 1968; Poinar & Hansen, 1983) is required.

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