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A study of corpuscular DNA and midgut gland occupancy by putative symbiotic elements in *Pomacea canaliculata* (Caenogastropoda, Ampullariidae)

I.A. Vega[1], C. Gamarra-Luques[1], E. Koch[1], L.E. Bussmann[2], and A. Castro-Vazquez[1]*

[1]Laboratory of Physiology (IHEM-CONICET), Department of Morphology and Physiology, University of Cuyo, Casilla de Correo 33, M5500 Mendoza, Argentina, Tel. +54-261-413-5006 or 449-4001, Fax. +54-261-449-4117, Emails. ivega@fcm.uncu.edu.ar, cgamarra@fcm.uncu.edu.ar, ekoch@fcm.uncu.edu.ar and acv@fcm.uncu.edu.ar; [2]Institute of Biology and Experimental Medicine-CONICET, Obligado 2490, C1428ADN Buenos Aires, Argentina, Tel. +54-11-4783-2869 or -4786-2564, Fax. +54-11-4786-2564, Email. bussmann@dna.uba.ar

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Abstract

Morphology of pigmented corpuscles (C and K types) in the midgut gland of *Pomacea canaliculata* has suggested that they may be morphotypes of a prokaryotic symbiont. Both corpuscles were isolated from midgut gland tissue to (1) quantify gland occupancy in different experimental conditions and (2) determine and preliminarily characterize DNA present in them. Pigmented corpuscles occupied 8.2–11% of the gland, and this percentage did not vary significantly according to sex or diet (either a mixed or a paper-only diet, during eight weeks). DNA was detected in both corpuscular types. DNA content in C corpuscles was estimated as 57 fg/corpuscle. Determination of the DNA/protein ratio in lysates of C corpuscles yielded values similar to those of bacterial cells used as controls (*Escherichia coli*, *Synechococcus* sp.). The DNA/protein ratio in K corpuscles, however, was much lower than in C corpuscles, probably owing to the high protein content of K corpuscles' envelop. PCR amplification of a ~1,500 bp fragment corresponding to the 16S rRNA gene was obtained when using template DNA extracted from both C and K corpuscles, as well as from an *Escherichia coli* culture used as control. The current results are consistent with the idea of a bacterial nature of C and K corpuscles.

Keywords: Molluscs, Gastropoda, applesnails, endosymbiosis, bacteria

1. Introduction

Pomacea canaliculata (Lamarck, 1822) belongs to a family of limnic gastropods widely distributed in tropical, subtropical and temperate zones over the Old and New World (Hylton Scott, 1957; Berthold, 1989). It occurs mainly in lentic habitats throughout the lower Amazon basin and the Plata basin (Hylton Scott, 1957; Martín et al., 2001). This Neotropical species has been introduced (ca. 1980) to several Asian countries for aquaculture, and has become a serious pest for rice crops (Halwart, 1994; Cowie, 2002).

P. canaliculata has been reported to host a variety of organisms, including some ciliates (Gascón, 1975), a rotifer (Gamarra-Luques and Castro-Vazquez, unpublished findings), arthropods (Vidrine, 1996; Gamarra-Luques et al., 2004) as well as some platyhelmynths (Damborenea, 1998; Damborenea and Cannon, 2001; Hamann, 1992; Keawjam et al., 1993), nematods (Leon-Dancel, 1970) and annelids (Damborenea and Gullo, 1996).

The midgut gland (MGG) of this snail bears numerous pigmented corpuscles that have been considered as digestive-excretory bodies (Andrews, 1964). We have proposed recently (Castro-Vazquez et al., 2002; Koch et al., in press) that they may be indeed morphotypes of a prokaryotic symbiont. Two distinct types (namely C and K corpuscles), as well as intermediate forms, may be recognized as originating within alveolar cells of the midgut gland of this snail and which are later eliminated in the feces. Typical C corpuscles are rounded, 14 μm width, granule-containing bodies, which are encased in an electron dense wall. They sometimes contain inner membranes also. K corpuscles, on their part, are dark brown, either oval or club shaped bodies (36 μm length, 14 μm width) which appear made of

*The author to whom correspondence should be sent.

electron dense lamellae surrounding a core of coarse granules.

In the present work we have isolated both types of corpuscles from MGG tissue, and this has permitted us: (1) to estimate the extent of the MGG occupied by these corpuscles (in both sexes, and in animals under different diets), (2) to quantify the corpuscular DNA content and DNA/protein ratio, and to compare the latter with that of other prokaryotic and eukaryotic cells, and (3) to amplify a 1500 bp DNA fragment using PCR primers designed to encompass the bacterial sequence encoding for 16S rRNA, and template DNA extracted from both C and K corpuscles.

2. Material and Methods

Animals

Mature males and females from a cultured strain of *Pomacea canaliculata* were used. The original stock was collected at the Rosedal Lake (Palermo, Buenos Aires, Argentina) and voucher (alcohol preserved) specimens of the original population and of the cultured strain were deposited at the collection of Museo Argentino de Ciencias Naturales (Buenos Aires, Argentina; lots MACN-In 35707 and MACN-In 36046, respectively). Temperature was regulated at 23–25°C and artificial lighting was provided 14 hs per day. Unless otherwise indicated, animals were fed *ad libitum* on a mixed diet made of lettuce, rodent food pellets and toilet paper. Sex was determined externally by the shape of the operculum (Cazzaniga, 1990) and confirmed at sacrifice.

Method for the isolation of C and K corpuscles from the MGG

A method involving osmotic lysis of glandular cells and their organelles, and which is followed by sequential sedimentations of the osmotic resistant corpuscles, was developed (Fig. 1). The procedure involved the following steps (all procedures were done in a water bath at 4°C):

Step 1

The MGG was dissected out, weighed and homogenized in a glass homogenizer, in 4 ml of TE-Az buffer (Tris 10 mM, EDTA 1 mM, 0.1% sodium azide; pH=7.4) per gram of tissue. The homogenate was cloth filtered and centrifuged at 750 g for 10 min and this first supernatant was discarded. The precipitate was dispersed in TE-Az buffer to a 5 ml volume, and left to sediment in the cold water bath for 30 min. Thus, one precipitate and one supernatant were obtained at the end of Step 1, and were processed separately afterward (the precipitate was much enriched in C and K corpuscles, while the supernatant still contained C corpuscles).

Step 2

The precipitate obtained in Step 1 was dispersed again in TE-Az buffer to a 5 ml volume and left to sediment for 60 min. The supernatant obtained in Step 1 was made up with TE-Az buffer to a 5 ml volume and left to sediment for 60 min, so that one precipitate and one supernatant were obtained. Thus, 2 precipitates and 2 supernatants were obtained at the end of Step 2, and each one of them were processed separately afterward.

Step 3

The precipitates obtained in Step 2 were dispersed to a 5 ml volume and left to sediment for 15 min, while the supernatants obtained in Step 2 were made up to a final volume of 5 ml and also left to sediment for 15 min. Thus, 4 precipitates and 4 supernatants were obtained at the end of Step 3, to be processed separately afterward.

Step 4

The precipitates obtained in Step 3 were again dispersed to a 5 ml volume and left to sediment for 15 min, while the supernatants obtained in Step 2 were again made up to a final volume of 5 ml and also left to sediment for 15 min. Thus, 8 precipitates and 8 supernatants were obtained at the end of Step 4.

Step 5

The precipitates of the tubes labeled 1–4 in Fig. 1 were pooled, dispersed in 40 ml of TE/Az buffer, and left to sediment for 15 min. The supernatant was discarded and the procedure was repeated thrice. The precipitate was dispersed in 1 ml of TE/Az buffer, a 5 µl sample was taken for microscopic control of the fraction, and the rest was centrifuged at 750 g for 5 min; the precipitate thus obtained (fraction K) was drained, weighed and frozen. Precipitates labeled 5–8 (containing a negligible amount of C corpuscles mixed with some small sized K corpuscles) were discarded.

The 8 supernatants obtained at the end of Step 4 were pooled and made up to 40 ml of TE buffer (Tris 10 mM, EDTA 1 mM; pH=7.4), and centrifuged at 750 g for 5 min. The supernatant was discarded and the procedure was repeated thrice. The precipitate was dispersed in 1 ml of TE buffer, a 5 µl sample was taken for microscopic control of the fraction, and the rest was centrifuged at 750 g for 5 min; the supernatant was discarded and the precipitate thus obtained (fraction C) was weighed and frozen.

Microscopic control of corpuscular fractions

A 5 µl aliquot of each sample resuspended corpuscular fraction was observed unstained under a bright field microscope (×400 magnification). The numbers of both C and K corpuscles were counted in a total of 100 microscopic

fields (50 on each diagonal line of a 22 mm square cover slip), and results were expressed as percent of each type of corpuscle in the obtained fraction.

Electron microscopy

Samples of the obtained C and K fractions were examined under transmission electron microscopy. They were fixed in a 4% paraformaldehyde-2.5% glutaraldehyde mixture in 0.1 M sodium phosphate buffer, pH 7.4 (SPB) for five hours and washed three times (10 min each) with the same buffer. Then the fractions were postfixed overnight with osmium tetroxide 1% in SPB, stained with uranyl acetate 2% during 45 minutes, and then dehydrated via graded ethanol and acetone.

Finally, the fractions were embedded in Spurr's resin. Ultrathin sections were obtained with a diamond knife. For topographic orientation, 1 μm sections were stained with 1% toluidine blue.

Extent of the MGG which is occupied by C and K corpuscles

The isolation procedure was used to estimate the extent of the MGG that is occupied by these corpuscles. Also, we explored if the amount of corpuscles could be modified in animals fed only on paper for a rather long period (60 days), as one could expect if the corpuscles were of dietary origin (e.g., as if they were partly digested chloroplasts). For such purposes, snails raised on the mixed diet mentioned above were drained, weighed and allocated to four groups according to their sex and to the *ad libitum* diet they received during the following 60 days: (1) males that continued to be fed on the mixed diet mentioned above; (2) male snails fed only on toilet paper during the experimental period; (3) females fed on the mixed diet during the experimental period; (4) females fed only on toilet paper during the experimental period. The aquaria were 33.5 cm × 22 cm × 11.5 cm, in which water level was kept approximately 5 cm deep, and

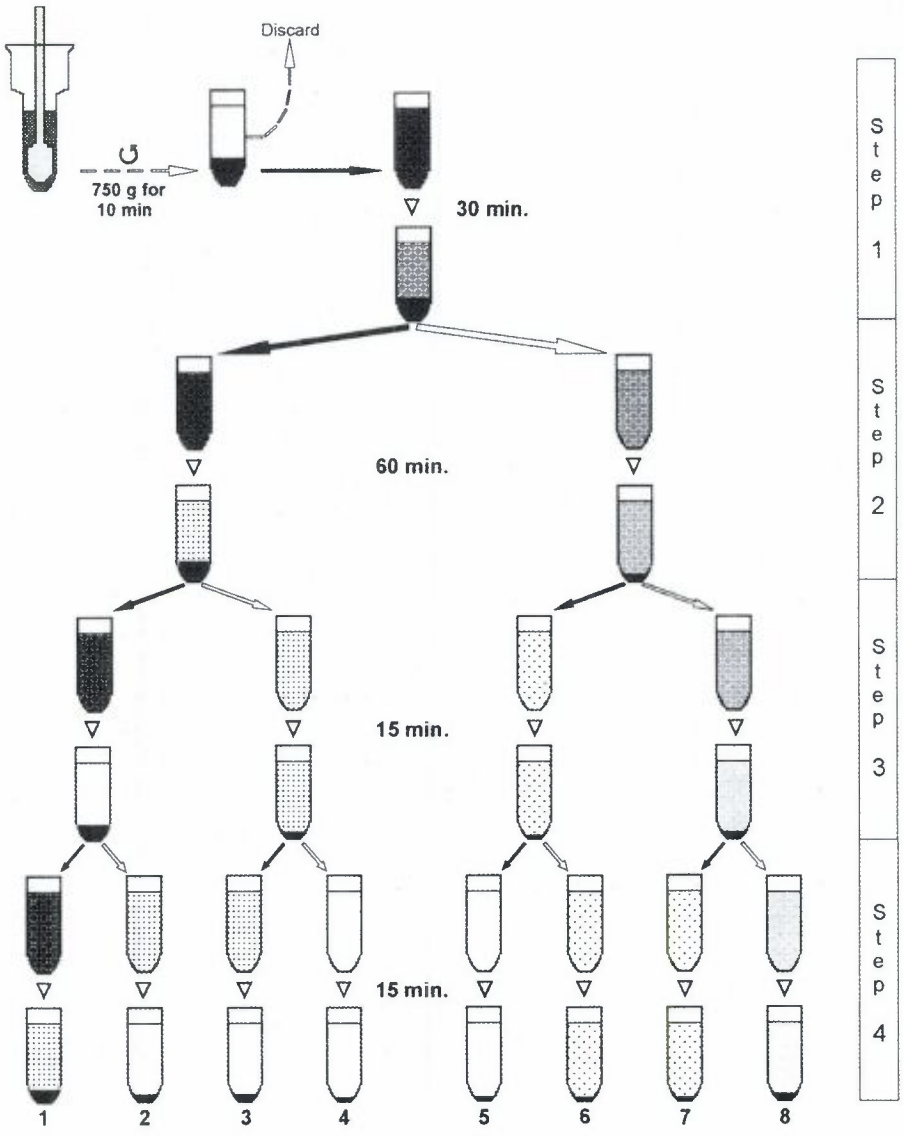

Figure 1. Procedure to isolate C and K corpuscles from midgut gland homogenates. Continuous black arrows indicate that the precipitate was resuspended in buffer. White arrows indicate that the supernatant was made up to the initial volume. White triangles indicate that the material (either the resuspended precipitate or the supernatant made up to the initial volume) was allowed to sediment during the indicated time. In step 5, precipitates from tubes 1–4 were pooled and washed to obtain fraction K. On their part, supernatants from tubes 1–8 were pooled, centrifuged (750 g, 5 min) and washed to obtain fraction C. Precipitates from tubes 5–8 were discarded.

ten animals of a single sex were placed in each. At the end of the experimental period (60 days), the snails were drained (after cooling them down in 4°C water during 10 min), and immediately weighed and sacrificed, and the MGG was dissected out, weighed, and processed to obtain the C and K fractions, according to the procedure described above. The extent of occupancy of the MGG by C and K corpuscles was calculated as mg of each type of corpuscles per gram of tissue.

DNA content in C corpuscles

To estimate mean DNA content in C corpuscles, the C fraction was obtained from MGG tissue as described above, and the corpuscles were resuspended in SPB and two 5 µl aliquots of the suspension were used for duplicate counting of corpuscles, while the rest was homogenized with an Ultraturrax homogenizer (10 min at 4°C). DNA was measured in the homogenates according to the *bis*-benzimide method (Labarca and Paigen, 1980) using salmon sperm DNA as standard. Since not all C corpuscles present in the samples were homogenized, the number of C corpuscles remaining after homogenization was substracted from the initial number. A mean of 70.2±7.4% of C corpuscles (N=11) was dissolved during homogenization. Results were expressed as fg of DNA per corpuscle.

Comparison of the DNA/protein ratio in C and K corpuscles and in other prokaryotic and eukaryotic cells

Both C and K corpuscles were isolated (as described above) from MGG tissue of animals of both sexes and that were fed on the mixed diet. *Escherichia coli* cells were obtained by centrifugation of an 8 hours culture at 37°C (Luria Bertani medium). *Synechococcus* sp. cells (Cyanobacteria, Chroococcales) were obtained from a long term "unialgal", non-axenic culture in aquarium water at room temperature. In both cases, bacterial cells were washed thrice in SPB and each time recovered by centrifugation at 10,000 g for 5 min; the final pellet was resuspended, aliquoted and frozen at –70°C until assayed. Human leucocytes were obtained from heparinized venous blood of an adult male. Blood samples were allowed to sediment at 37°C for 5 hours and then both the plasma and the whitish segment between plasma and erythrocytes were aspirated with a microsyringe. The aspirate was microscopically controlled for erythrocytes contamination, and then was aliquoted and centrifuged at 4°C (120 g for 5 min); the pellet was washed and frozen as indicated for bacterial cells.

All frozen pellets were lysed by incubation in 0.2 N sodium hydroxide at 50°C during 30 min. DNA content was measured by the *bis*-benzimide method. Protein content was measured by the method of Lowry et al. (1966) using bovine albumin as standard.

Standard curves for both methods were also run after incubation at 50°C during 30 min, with or without the replacement of the sample buffer by 0.2 N sodium hydroxide, to control for a presumptive bias of determinations in the hydroxide treated samples, but no such bias was found. Additional procedures were run for both the C and K fractions, to control for a possible adsorption of any DNA to the external surface of corpuscles: (1) both DNA and proteins were determined (and found undetectable; N=4) in the supernatant after washing the corpuscles, and (2) corpuscular DNA content was not affected by previous deoxyribonuclease I (DNAse I) treatment of corpuscular samples (N=8). For the latter purpose, 375 units (6.4 µg) of DNAse I (Amersham Pharmacia Biotech) were added to each corpuscular sample and incubated at 37°C during 15 min and then washed twice in SPB.

Polymerase chain reaction (PCR) mediated amplification of DNA encoding for bacterial 16S rRNA

The C and K fractions from 1–2 glands (obtained as already described) were pooled and washed thoroughly in buffer and pelleted before template DNA extraction (experimental samples). *E. coli* was cultured overnight as described above, and pelleted before extraction (control samples).

One of the following protocols was applied to them: (1) they were lysed and extracted using the phenol-chloroform-isoamylic alcohol method (Sambrook et al., 1989); (2) they were treated according to the protocol for DNA extraction and purification of QIAgen GmbH (D-40724, Hilden) which includes proteinase K treatment, and passage through an adsorption resin column, from which purified DNA was eluted later; (3) in this third protocol, both C and K fractions (but not *E.coli* cells) were pretreated to remove any residual bacterial contamination.

For such purpose, C and K corpuscles were treated before homogenization with lysozyme (20 mg/ml, 37°C, 30 min), sodium dodecyl sulfate solution (2% v/v, 37°C, 30 min), and DNAse I and RNAse H (10 µg/ml of each enzyme, 37°C, 30 min); the corpuscles were washed twice in 10 mM Tris-HCl buffer (pH 7) before and after DNAse/RNAse treatment; afterwards, they were treated with proteinase K (50 µ/ml, 56°C, 120 min), washed twice and pelleted by centrifugation. After this differential treatment of pigmented corpuscles, template DNA was extracted form both control and experimental samples using the SoilMaster™ DNA Extraction Kit, whose protocol is designed to remove PCR inhibitors, and which includes passage through a resin exclusion column.

Afterwards, oligonuclotide primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525r (5'-AAGGAGGTGWTCCARCC-3'; Weisburg et al., 1991) corresponding to highly conserved sequences of bacterial 16S rRNA gene, and encompassing most of this gene sequence, were used for PCR. The reaction was performed in a total volume of 50 µl, containing template DNA

dilutions, 0.2 μM primers (Invitrogen), 0.4 mM nucleotides mix (dNTPs, Promega) and 1 U Taq polymerase (Promega). The PCR program used included an initial denaturation step at 94°C for 3 min that was followed by 30 cycles each one including denaturation at 94°C (30 s), annealing at 60°C (30 s) and extension at 72°C (2 min). The reaction products were separated in 1.5% agarose gel electrophoresis and stained with ethidium bromide.

Statistical analysis

The distribution of variables was evaluated by Kolmogorov-Smirnow's normality test. Differences between two groups were analyzed with Student's t-test. Multigroup comparisons were made using ANOVA I, and the Newman-Keuls test as a *post-hoc* analysis. In all cases, significance level was fixed at $P<0.05$.

3. Results

Isolated C and K corpuscles

Microscopic control of both C and K fractions yielded 91.6±0.8% of C corpuscles for the C fraction (mean±SEM, N=20) and 99.4±0.3% of K corpuscles for the K fraction (N=20). K corpuscles contaminating the C fraction were mainly small sized, loosely packed corpuscles, representing a likely transition from one type of corpuscle to the other.

Control of the C fraction under transmission electron microscopy showed well preserved corpuscles (Fig. 2), similar to those normally found within glandular cells (Koch et al., in press). Most of these bodies are spherical, contain both small and large granules, and appear lined by an outer membrane and electron dense wall which usually overshadows the outer membrane (Fig. 2B). The C fraction also contains a limited amount of empty microsomal vesicles, which are probably the result of the sealing of membranes from the osmotically lysed cells. Electron microscopy of the K fraction showed both solid and multilamellar K corpuscles.

Extent of the MGG which is occupied by C and K corpuscles

Body mass (drained) of the snails was not significantly affected by either sex or feeding (ANOVA I, Table 1). MGG mass was significantly smaller in males than in females, and it was reduced in paper fed animals of both sexes as compared with control snails on the mixed diet (ANOVA I, Newman-Keuls test). The mass of both C or K corpuscles per animal, however, was not significantly affected by either sex or diet (ANOVA I, Table 2).

DNA content and DNA/protein ratio in glandular corpuscles and in some prokaryotic and eukaryotic cells

Mean corpuscular DNA content could be determined only

Table 1. Body and midgut gland mass in snails of both sexes after 60 days of feeding on a mixed diet or on paper only.

	Mixed diet		Paper only	
	Males	Females	Males	Females
Body mass (g)	16.85±0.97 (N=18)	16.39±0.81 (N=13)	14.73±0.70 (N=13)	13.32±0.80 (N=14)
MGG mass (absolute, g)	0.81±0.04 ab (N=18)	1.09±0.06 ab (N=13)	0.49±0.03 ab (N=13)	0.71±0.04 ab (N=14)
MGG mass (% of body mass)	4.86±0.30 ab (N=18)	6.72±0.20 ab (N=13)	3.35±0.19 ab (N=13)	5.50±0.23ab (N=14)

Results are expressed as mean±SEM. N is number of cases. a: Significantly different from animals on the same diet but of the other sex (ANOVA I; Newman-Keuls test). b: Significantly different from animals of the same sex but on the other diet (ANOVA I; Newman-Keuls test).

Table 2. Amount of C and K corpuscles in snails of both sexes after 8 weeks of feeding on a mixed diet or on paper only.

	Mixed diet		Paper only	
	Males	Females	Males	Females
C corpuscles (mg per snail)	19±4 (N=18)	33±6 (N=13)	17±3 (N=13)	25±2 (N=14)
K corpuscles (mg per snail)	72±9 (N=18)	61±11 (N=13)	46±8 (N=13)	53±9 (N=14)
C corpuscles (mg per g of gland tissue)	22±3 (N=18)	30±6 (N=13)	36±4 (N=13)	33±3 (N=14)
K corpuscles (mg per g of gland tissue)	82±10 (N=18)	62±14 (N=13)	93±15 (N=13)	74±10 (N=13)

Results are expressed as mean±SEM. N is number of cases per group. There were no significant differences between groups (ANOVA I).

in C corpuscles and was 57.1±9.2 fg/corpuscle (mean ± SEM, N=11). The range was 14–97 fg/corpuscle. Accurate estimations of corpuscular DNA content in the K fraction was not possible, since these corpuscles could not be adequately dissolved during homogenization.

Therefore, a method of lysis in hot sodium hydroxide, followed by the determination of the DNA/protein ratio in lysates, appeared as a more suitable procedure to compare DNA concentration in cells that could be homogenized with a very variable efficiency.

In such conditions, the DNA/protein ratio in lysates of the C fraction was significantly higher than in those of the K fraction (44.1±7.9 µg/mg, N=26, and 12.8±3.0 µg/mg, N=26, respectively; Student's t-test).

Also the DNA/protein ratio in lysates of C corpuscles was compared with that of some prokaryotic (*Escherichia coli*, *Synechococcus* sp.) and eukaryotic cells (human leucocytes). Only human leucocytes yielded a significantly lower DNA/protein ratio (as compared with any of the other studied cells, Fig. 3, ANOVA I, Newman-Keuls test).

PCR mediated amplification of DNA encoding for bacterial 16S rRNA

DNA extracted from *E.coli* cultures, both with phenol-chloroform-isoamylic alcohol method and with the QIAgen protocol, yielded single bands of approximately 1,500 bp when used as template for PCR, no matter the extraction

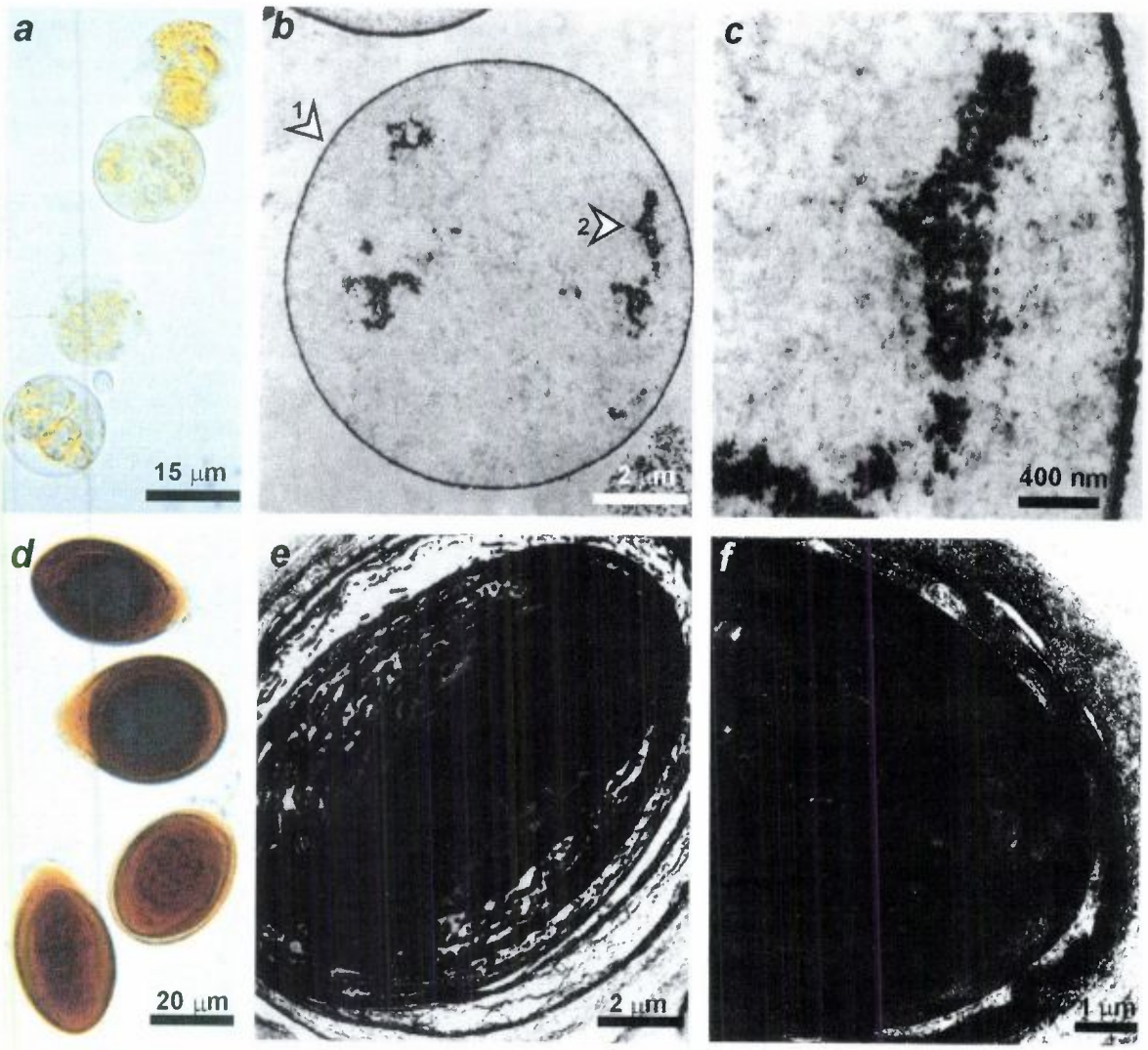

Figure 2. Panels a and d are micrographs of unstained preparations of isolated C and K corpuscles, respectively. Panels b and c are transmission electron micrographs from an isolated C corpuscle: arrow [1] indicates the external electron dense wall, while arrow [2] points to a cluster of inner granules, which appears enlarged in panel c. The lower panels e and f are electron micrographs of two K corpuscles showing concentric lamellae surrounding a more compact core.

and purification protocol used. However, no DNA amplification occurred when C and K corpuscular DNA was extracted either by the phenol-chloroform-isoamylic alcohol method or the QIAgen protocol. In fact, total inhibition of the PCR was also observed when 10 ng of corpuscular DNA were added to *E. coli* template DNA preparations. This suggested us that a material that was co-purified with corpuscular DNA was inhibiting PCR, and we looked for a method to remove such inhibitors. Finally, when template DNA from both C and K corpuscles was prepared according to a protocol designed to remove PCR inhibitors (SoilMaster™ DNA Extraction Kit) the amplification of 1,500 bp bands was observed (Fig. 4). Preparation of *E. coli* template DNA with the SoilMaster™ protocol also yielded a band of similar molecular size (Fig. 4).

4. Discussion

Pigmented corpuscles in the MGG of gastropods have been known to occur since the 1800's. They have been regarded as containing "chlorophyllous pigments" derived from food, and/or as having an excretory function (see MacMunn, 1883, 1900, for earlier references). MacMunn (1883) was also the first to note that they "remind one strongly of unicellular algae", but he later abandoned this view (MacMunn, 1900). Meenakshi (1955) first noticed them in an ampullariid snail (*Pila virens* Olivier, 1804), and Andrews (1964) attributed them a digestive-excretory function in *Pomacea canaliculata*. Notably, pigmented corpuscles occupy about 8.2–11% of the glandular mass in *P. canaliculata*, and these figures did not differ significantly whether the animals were fed on a mixed diet or on a paper-only diet during 8 weeks. The present paper is part of a broader program aimed to examine the possible symbiotic nature of these corpuscles (Castro-Vazquez et al., 2002; Koch et al., in press).

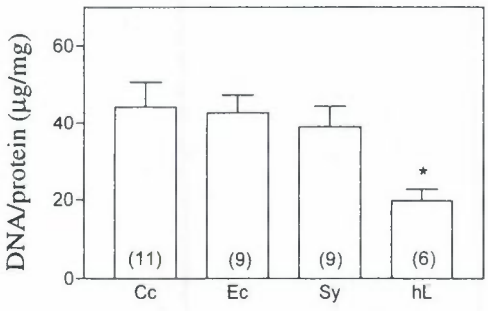

Figure 3. Comparison of the DNA/protein ratio in C corpuscles (Cc) and in some prokaryotic and eukaryotic cells: *Escherichia coli* (Ec) and *Synechococcus* sp. (Sy) and human leukocytes (hL). Bars indicate means ± SEM. The number of cases of each group is shown in brackets. The asterisk indicates significantly different from all other groups (ANOVA I, Newman-Keuls test).

In that context, the PCR amplification of 1,500 bp fragments encompassed by primers corresponding to the 1,500 bp 16S rRNA gene is strong evidence in favor of the bacterial nature of both C and K corpuscles. Even though these molecular data should not be necessarily interpreted as C and K corpuscles being morphotypes of the same organism, the likelihood of their identity is suggested by the observed morphological transition from one morph to the other (Koch et al., in press). However, comparison of DNA sequences from both corpuscular types will be needed to definitely clarify this point.

Molecular evidence in favor of the bacterial nature of C corpuscles is in keeping with the ultrastructural appearance of these bodies, in that they are anuclear, granule containing bodies which are encased within an electron dense wall (Koch et al., in press). Although they would be rather large bacteria (14 µm), their size is within the range of unicellular cyanobacteria (Castenholz and Waterbury, 1989). However, their ultrastructural features are quite different from typical cyanobacterial cells, particularly in that they lack thylacoids (Koch et al., in press).

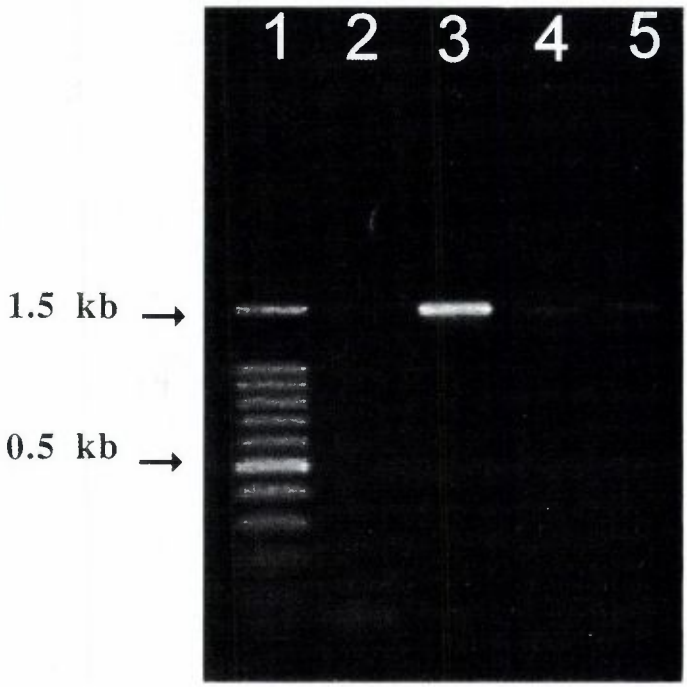

Figure 4. PCR mediated amplification of most of the sequence of the 16S rRNA gene, using template DNA extracted from C and K corpuscles isolated from the midgut gland and from an *E. coli* culture. The extraction/purification protocol used (SoilMaster™) was designed to remove PCR inhibitors. The PCR products were separated in 1.5% agarose electrophoresis and stained with ethidium bromide. The different lanes are: (1) molecular weight standards, ranging 100–1,500 bp; (2) negative control reaction without template DNA; (3) positive control reaction with *E. coli* template DNA; (4) reaction using template DNA extracted from C corpuscles; and (5) reaction using template DNA from K corpuscles.

The estimation of 57 fg DNA/corpuscle that is made in this paper is above the range of known bacterial genome sizes (Cavalier-Smith, 1982; Cole and Saint-Girons, 1999; Gregory, 2001), which span from 0.64 fg (*Mycoplasma genitalium*, Mycoplasmales) to 10.56 fg (*Nostoc punctiforme*, Cyanobacteria). It should be noted, however, that current estimations of the DNA content of C corpuscles should be averaging corpuscles with varying numbers of genome copies, and therefore, they are not indicative of the genome size of the putative symbiont.

Also, determination of DNA/protein ratio in sodium hydroxide lysates yielded similar ratios for C corpuscles and for *Escherichia coli* and for *Synecococchus* sp. cells. As expected, human leukocytes yielded a significantly smaller ratio, since eukaryotic cells have generally lower DNA/protein ratios than prokaryotic cells (e.g., Alberts et al., 1994). The much lower DNA/protein ratio found in sodium hydroxide lysates of K corpuscles may be due to two reasons: (1) the high protein content of the coat, and (2) to an underestimation of their DNA content, since there is evidence that some substance present in K lysates quenches the fluorescence of the *bis*-benzimide/DNA complex (Castro-Vazquez et al., 2002). Further studies will be needed to precisely identify these putative prokaryotic symbiont/s in the midgut gland of *P. canaliculata*.

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