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Workshop: Biology of Ampullariidae

Facultative and obligate symbiotic associations of *Pomacea canaliculata* (Caenogastropoda, Ampullariidae)

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Pomacea canaliculata (Lamarck 1822) occurs mainly in lentic habitats throughout the lower Amazon basin and the Plata basin (Hylton-Scott, 1958; Martín *et al.*, 2001). This Neotropical species has been introduced (ca. 1980) to several South East Asian countries for aquaculture, and has become a serious pest for rice and other crops (Halwart, 1994). Many Pacific islands are currently included in the invaded area (Cowie, 2002).

In a previous previous publication we have presented preliminary evidence for a symbiotic association of *Pomacea canaliculata* and of other Neotropical Ampullariidae with a large and pigmented prokaryotic symbiont within specific cells of the midgut gland of this snail (Castro-Vazquez *et al.*, 2002). The current paper overviews the diversity of symbiotic associations of *P. canaliculata*, and updates the information on the prokaryotic symbiont, which is most likely a cyanobacterium.

What do we put in the term "symbiosis"?

The development of symbiosis theory has been a complex one, as Sapp (1994) has masterfully depicted, and this is many times reflected in a conflicting use of terms. What is now called "symbiosis" was conceptually postulated by the first time in 1868, by the Swiss botanist Simon Schwendener (1868), who interpreted the lichens as modified fungi that keep a green algae[1] "in slavery". For this special parasitic[2] relationship, Johannes Reinke (1873) suggested the use of *consortium*, a word that is in use even today, and another German lichenologist, Albert Bernhard Frank (1877), coined the term *Symbiotismus.*[3] Even though his studies were well known and his treatise on Botany (Frank, 1892) was widely used, credit for coining the term was (and

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[1] By 1868, the Algae were including the Cyanobacteria.

[2] The term "parasite" (and derived words) has been used in European languages since ancient times. For the Greeks, παρασιτος (*parasitos)* was that who eats besides someone else, a concept akin to that of the Latin word *commensal*, i.e., that one who eats at the same table, or even who merely seats besides someone else; derived (and more specific) uses of παρασιτος referred to the citizens that were fed at the expense of the state, and eventually to macroscopical parasites of man and animals, in a strict biological sense.

is) generally given to Heinrich Anton de Bary, who first used the term symbiosis (*Symbiose*) in his address to a joint meeting of German naturalists and physicians (de Bary, 1879). He defined it as "the living together of unlike named organisms", and said that "parasitism, mutualism, lichenism, etc., are each special cases of that one general association for which the term symbiosis [*Symbiose*] is proposed as the collective name".[4]

Buchner (1921) studied the microbial symbionts of aphid insects and concluded that the development of such cells, tissues and organs as the aphid mycetocytes and mycetosomes could not occur without having profoundly altered the physiology and even the habits of the hosts. This was a seed notion that symbiotic relationships are the outcome of a co-evolutive process. Nuttall (1923) and Meyer (1925) elaborate this argument and conceived the origin of symbiosis through a preliminary stage of parasitism on the part of one of the associated organisms, and the solution of the conflicts between them, in the course of time, by their mutual adaptation. Both species have to alter their physiology and even their habitats, and –which is worst– all the process is, as normal in Evolution, open-ended: co-evolution may imply a one-to-one gene adjustment every time the host or the symbiont get some significant mutation (Ridley, 1996).

It has been early acknowledged that the difference between parasitism and mutualism might be blurred, not only in the course of evolution but also in the host's lifetime (Nuttall, 1923; Meyer, 1925). As an example of the latter, *Candida albicans*, a harmless inhabitant of the human skin and intestine, causes lesions when either the associated bacterial biota or the immune status of the host changes. Even cyanolichens, generally quoted as prime examples of mutualism between eukaryotic and prokaryotic organisms, do not show anything but a continuous cline of possibilities from parasitic to mutualistic interactions (Rikkinen, 2002).

For these reasons, many authors have come to use the term symbiosis as encompassing parasitic, commensal and mutualistic interactions, i.e., as a comprehensive concept that does not consider the role which the two individuals play but is based in the mere coexistence, as Frank (1877) proposed more than a century ago. More recently, the concept was extended to include some associations involving not whole cells but dietary chloroplasts (Kawaguti and Yamasu, 1965; Taylor, 1968; Greene, 1974; Trench, 1975), as found in the cells of the midgut gland in elysioid opistobranch slugs (a special case of symbiosis referred to as "kleptoplasty", Gilyarov, 1983; see also Rumpho *et al.*, 2000, for a recent review). A similar association has only recently been reported for the Argentinean marine slug *Elysia patagonica* (Muniain *et al.*, 2001). In fact, the consequences of such acquisition of chloroplasts may bear interesting parallels with the apparently cyanobacterial-gastropod symbiosis that will be commented on later in this paper.

For the sake of clarity, we will briefly and precisely define some terms related to the concept of "symbiosis", as they will be used in this review: (1) In a symbiotic association (sometimes, also called consortium) the largest organism is named the host, while the smaller is named the symbiont. (2) Obligate (as opposed to facultative) symbioses refer to associations involving symbionts found in all individuals of the host species. (3) Epibiosis and derived terms are used when the symbiont lives on the external parts of the host (for *P. canaliculata*, the periostracum and the operculum). (4) Endosymbiosis (and derived terms) refers to a consortium in which the symbiont lives within the limits of the host's body (also including cavities communicated to the exterior, as the gut and mantle cavity). (5) Endocytobiosis (and derived terms) refers to an intracellular endosymbiosis. (6) Cyanobiont refers to a cyanobacterium involved in a symbiosis.

A survey of symbiotic associations with Eukarya

Epibiotic associations

In a study on *P. canaliculata* individuals, collected in both lotic and lentic habitats in the surroundings of the middle Paraná river, Di Persia and Radici de Cura (1973) described a diverse community of epibiotic organisms covering the periostracum (and to a lesser extent, the operculum) of this species. Also, they demonstrated (1973) that those epibiotic community is different between dead and live snails (diversity and

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[3] "We must bring all the cases where two different species live on or in one another under a comprehensive concept which does not consider the role which the two individuals play but is based in the mere coexistence and for which the term symbiosis [*Symbiotismus*] is to be recommended" (Frank, 1877).

[4] As for "parasitism", "symbiosis" has also ancient roots: συμβιωτης (*symbiotes*) was said of who was living with someone else, or of who was having a favorite relation with someone. Συμβιωτικος (*symbiotikos*) was applied to anything related to life in common.

quantity), suggesting that the movement of the host is necessary to maintain the community of epibiotic organisms). Taxonomically equivalent communities were also observed in *Pomacea insularum* and *Pomacea scalaris*, although the epibiotic mat was thicker in *P. canaliculata* and *P. insularum* than in *P. scalaris* (a fact likely related to the smoother surface of the periostracum in the latter species). The composition and stratification of the epibiotic community may be outlined as follows: (1) a rather thick mat, predominantly composed by the chlorophytes *Stigeoclonium sp.* and *Oedogonium sp.* (Oedogoniaceae), together with species in the genera *Spirogyra* and *Mougeotia* (Zygonemataceae), *Scenedesmus* (Scenedesmaceae) and *Ankistrodesmus* (Oocystaceae); chlorophytes were followed in abundance by chrysophytes in the genera *Gomphonema* (Gomphonemaceae, living on *Stigeoclonium*) and *Navicula spp.* (Naviculaceae); sessile ciliates (*Vorticella campanula*, Vorticellidae, and *Epistylis plicatilis*, Epistylidae) also occur in this basal substratum were also composing the algal mat; (2) a diverse biota of motile organisms inhabiting the predominantly algal mat, including euglenophytes (*Trachelomonas spp.* and *Phacus spp.*), a variety of ciliates in the genera *Coleps* (Colepidae), *Didinium* (Didiniidae), *Chilodonella* (Chilodonellidae) and *Codonella* (Codonellidae), rotifers (genera *Rotaria,* Philodinidae, and *Keratella* and *Brachionus*, Brachionidae), nematodes (genus *Actinolaimus,* Actinolaimidae), leeches (*Helobdella ampullariae*, Glossiphoniidae), oligochaete (*Chaetogaster limnaei*, Naididae), aphanoneurans (*Aeolosoma spp.*, Aeolosomatidae) and dipterans larvae (*Chironomus sp.*, Chironomidae). Cazzaniga (1988) also observed an ectoproct (*Hyalinella vahiriae*) living in the suture of *P. canaliculata*, and Dreher Mansur *et al.*, (2003) and Darrigran and Damborenea (2005) have recently added the exotic golden mussel *Limnoperna fortunei* (Mytilidae) to the list of frequently found epibionts.

Symbiotic animals in the mantle cavity

A variety of organisms have been found dwelling within the mantle cavity of *P. canaliculata*. However, it should be noted that their diversity (even if we include those endosymbionts in other snail's organs) does not parallel that of the epibiota, which may indicate some selectivity on either the part of the host or of the symbionts. Some possibilities of selectivity on the part of the symbionts suggest themselves, e.g. that photosynthetic organisms would not be able to survive as endosymbionts in dark environments, or that sessile organisms will not found adequate surfaces for fixation. It is also possible that the mucous secretion and the water current in the mantle cavity may be unfavorable for the settling of many organisms.

Platyhelminthes, Turbellaria:

Temnocephala iheringi (Temnocephalidae) is the most common turbellarian in the mantle cavity of *P. canaliculata* and it also extends to the lung. The egg capsules are always laid over the periostracum, especially at the opening of the umbilicus and in the contact zone of the peristome with the suture. *T. iheringi* has also been found associated to other ampullariid species (*Pomacea haustrum*, *Pomacea lineata*, *Asolene platae* and *Pomella megastoma*, Damborenea and Cannon, 2001). This temnocephalid symbiont has been recorded from Brazil, Argentina and Uruguay, while other two species (*Temnocephala haswelli* and *Temnocephala rochensis*) have only been reported from Uruguay.

High prevalence values of *T. iheringi* (92-100%) were found in two *P. canaliculata* populations: Bagliardi beach, on the southwest bank of the Plata river (Damborenea, 1996) and Regatas Lake, Palermo, Buenos Aires city (Table 1). Abundance was about 14 temnocephalids *per* snail in the Bagliardi beach study (range: 0-167; n=303), and no significant differences in abundance were found between male and female hosts (Damborenea, 1996). Both abundance and aggregation in the mantle cavity were higher in late spring and summer (Damborenea, 1998). Although *T. iheringi* was also found associated to other ampullariid species (Damborenea and Cannon, 2001), stronger *Aeolosoma* association to *P. canaliculata* was apparent, since no temnocephalid worms were found in either *P. scalaris* (Damborenea, 1996; and Table 1) or *Asolene pulchella* (Table1). Nevertheless, this is not an obligate symbiosis for *P. canaliculata* either: in a study covering 20 habitats occupied by *P. canaliculata* in the province of Buenos Aires (Martín *et al.*, 2005) *T. iheringi* was found in only eight sites (three out of 13 lotic habitats, and five out seven lentic habitats). No information is available regarding the functional significance of this consortium.

Platyhelminthes, Trematoda:

Several trematode larval stages have been described associated to *P. canaliculata,* both found in the mantle cavity (cercariae and metacercariae).

Probably related rediae were found in the connective tissue of the midgut gland and gonad (see below). The identification of trematode species is based on the adult forms, which are usually found in vertebrates. Therefore, generic or specific identification of larval stages is often impossible, unless the entire life cycle could be studied.

Two types of cercariae have been found in the mantle cavity of snails, in both streams and lakes of the Buenos Aires province: schistosomatid cercariae have been found in the Luján river and on the Bagliardi beach (Damborenea, unpublished data); and a xiphidiocercaria has also been found in the same localities (Ostrowski de Nuñez, 1979). Metacercariae have also been described in the mantle cavity (*e.g. Echinostoma parcespinosum*, Martorelli, 1987). The metabolic, physiological and/or ecological interactions between these larval stages and their host are unknown.

Annulata, Hirudinea:

Five Glossiphoniid leeches have been found in the mantle cavity of *Pomacea canaliculata* collected on the Bagliardi beach (Plata river): *Helobdella ampullariae*, *Helobdella triserialis*, *Helobdella simplex*, *Helobdella adiastola* and *Gloiobdella michaelseni* (Damborenea and Gullo, 1996). As we have already mentioned, some individuals are also found on the operculum and periostracum.

All life stages of *H. ampullariae* (juveniles, adults and adults with both cocoons and broods) were found inside the snail, but they were never found free in the habitat (Damborenea and Gullo, 1996).

The reproductive season extends from December to June, i.e., overlapping to some extent the quiescent seasonal period of *P. canaliculata*, when the host is usually buried in the mud. The abundance of *H. ampullariae*

Table 1.

Symbiotic animals associated to *Pomacea canaliculata* and *P. scalaris* in Regatas Lake, Palermo, Buenos Aires city.

	Pomacea canaliculata				*Pomacea scalaris*			
	Periostracum and/or operculum	Mantle cavity	Perintestinal sinus	Prevalence %	Periostracum and/or operculum	Mantle cavity	Perintestinal sinus	Prevalence %
Helobdella ampullariae	0.6 ± 0.5 (0 – 6)	11.2 ± 2.2 (0 – 16)	0 (0 – 0)	90	0.1 ± 0.1 (0 – 1)	0 (0 – 0)	0 (0 – 0)	10
Temnochepala iheringi	0 (0 – 0)	17.4 ± 2.2 (12 – 25)	0.8 ± 0.4 (0 – 4)	100	0 (0 – 0)	0 (0 – 0)	0 (0 – 0)	0
Ozmana huarpium	0 (0 - 0)	5.5 ± 2.6 (0 – 16)	4.6 ± 7.9 (0 – 12)	100	0 (0 – 0)	0 (0 – 0)	0 (0 – 0)	0
Limnoperna fortunei	0.1 ± 0.4 (0 – 1)	0 (0 – 0)	0 (0 – 0)	10	0.1 ± 0.1 0 - 1	0 (0 – 0)	0 (0 – 0)	10

Results are expressed as both mean number of individuals *per* snail, ± SEM, and as the sample range (between brackets). Ten snails of each *Pomacea* species (as well as ten *Asolene pulchella* individuals) were collected in the Regatas Lake (Palermo, Buenos Aires city). No symbiotic animals were observed on or within *A. pulchella* individuals.

on *P. canaliculata* increases with the host size: 0-1 leeches were found in juvenile snails (<20 mm length), while up to 65 leeches were found in bigger snails, and the symbiont prevalence rose to 100%. Transmission of the symbiont probably occurs during copulation (Damborenea and Gullo, 1996). This leech has been also found as an endosymbiont to *Pomella megastoma* and *P. insularum,* Ampullariidae, and *Chilina fluminea*, Chilinidae (Ringuelet, 1945, 1985).

Both brooder adults and juveniles of the other four *Helobdella* species were only found during the spring and summer (Damborenea and Gullo, 1996), while *H. ampullariae* was also found in autumn and winter. No information is available of the functional significance of these consortia with *P. canaliculata*. They are not present in all populations of the snail, and therefore, should not be considered obligate symbiosis. The associations probably benefit the brooders and juveniles with food and protection.

Crustacea, Copepoda:

So far, only two copepod species have been described as endosymbiotic to freshwater invertebrates, and they both were found associated to *Pomacea* species: *Ozmana haemophila* (Cyclopoidea, Ozmanidae, Ho and Thatcher, 1989), which is associated to *P. maculata*, and *O. huarpium* (Gamarra-Luques *et al.*, 2004) which is associated to *P. canaliculata*.

O. huarpium was found in rather large numbers (about one hundred copepods per snail) in snails from both a cultured strain and from the locality of the strain's original stock (Palermo, Buenos Aires City). In spite of the large number of copepods, there was no evidence of tissue reactions or of any harm to the host. While *O. haemophila* was found restricted to the haemocoel of *P. maculata*, and occurring in rather small numbers (1-14 copepods per snail), *O. huarpium* (also found in the haemocoel) predominates in the penis sheath groove, the ctenidium and the mantle cavity, figuring in these pallial organs 63-65% of total mature forms. The sex ratio of the endosymbiont is skewed to the female side in these organs, specially in male hosts. Apparently, a special female tropism of *O. huarpium* for the male host's pallial organs might ensure interindividual transmission of the endosymbiont, which would be mainly transmitted during copulation.

All snails were bearing *O. huarpium* in the original study (Gamarra-Luques *et al.*, 2004). Interestingly, however, no copepods were found either in *P. scalaris* or *Asolene pulchella* that were collected in the Regatas lake (Palermo, Buenos Aires city) where all *P. canaliculata* were infested, indicating a specificity of the consortium between *P. canaliculata* and *O. huarpium* (Table 1). However, this is not an obligate symbiosis for the snail, since no copepods have been found in *P. canaliculata* individuals collected in four localities other than Palermo.[5]

Aracnida, Acari:

An unionicolid species, *Unionicola (Ampullariatax) ampullariae*, has been reported in the mantle cavity of both *P. canaliculata* and *P. insularum* (Vidrine, 1996). Di Persia and Radici de Cura (1973) also mention that up to 14 individuals may be found in large snails, and larvae, nymphs and adults may all of them be found in the mantle cavity. We have observed that they are not present in all populations of *P. canaliculata*, and therefore, they do not constitute an obligate symbiosis.

Symbiotic animals in the haemocoel and connective tissue

Platyhelminthes, Trematoda:

Two redia generations of *Echinostoma parcespinosum* were described in the connective tissue of the digestive gland and gonad of *P. canaliculata*. One to three cercariae were observed inside rediae of the second generation. In this case the snail is the first intermediate host of this parasite, whose adults are found in the intestine of the rails *Rallus maculatus* and *Rallus sanguinolentus* (Martorelli, 1987).

Rediae containing immature cercariae of *Dietziella egregia* (whose definitive host is the white-faced ibis *Plegadis chihi*) were observed just once (Digiani and Ostrowski de Nuñez, 2000). Some different but unidentified schistosomatid metacercariae have been frequently found in the pericardial cavity (Damborenea, unpublished data) and in the posterior renal cavity (*Dietziella egregia*, Digiani and Ostrowski de Nuñez, 2000).

The above mentioned studies were carried out within the native range of *P. canaliculata*, while Keawjam *et al.* (1993) have studied natural infestation of the snail in Thailand, an invaded area (Cowie, 2002).

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[5] The studied limnotopes where *O. huarpium* was looked for and not found were a fishing club near the town of Tunuyán (Mendoza), the banks of the Conlara river, near the town of Concarán (San Luis), the Bagliardi beach (on the Plata river) and the Tafí river (Tucumán).

They have found three different trematode metacercariae: amphistomes (from the foot), distomes (from the heart, kidney and foot muscle), and echinostomes (from the foot). The effect of these larvae on *P. canaliculata* was not assessed, but the authors suggest that some alteration in the reproductive efficiency might occur.

Crustacea, Copepoda:

As we already mentioned, *Ozmana huarpium* is not only found in the mantle cavity but also in the haemocoel of *P. canaliculata*, and it was occasionally found in hollow organs as the seminal vesicle, but not in the gut. It was never found in connective tissue.

Symbiotic protists and animals in the gut

Ciliophora:

Two species of large ciliates, *Parasicuophora ampullariarum* and *Parasicuophora corderoi* have been described as inhabiting the gut of *P. canaliculata* (Gascón, 1975). These ciliates are not found in all populations of the host, therefore should be classified as facultative symbioses.

Rotifera:

We have frequently observed a bdelloid rotifer (Gamarra-Luques and Castro-Vazquez, unpublished findings) in the gut content and feces of a cultured strain of *P. canaliculata*. This rotifer remains alive for at least several days outside the snail, i.e., in the aquarium sediments. A proper description of this association is wanting. Also, no studies of the distribution of this rotifer in the natural populations of *P. canaliculata* are available.

Nematoda:

Chao *et al.* (1987) have reported the experimental infection of *P. canaliculata* by *Angiostrongylus cantonensis*. This worm does not occur in the native range of *P. canaliculata*, but it does in the invaded territories. Snails may become infected as a consequence of ingestion of nematode eggs present in rodent fecal pellets. Man is infected by ingestion of third state larvae (Jindrak, 1975). This nematode is a common causative agent of human eosinophilic meningoencephalitis, a serious and frequently epidemic disease.

Platyhelminthes, Trematoda:

One species of Paramphistomidae, *Catadiscus pomaceae*, has been found in the intestine of *P. canaliculata* from a natural population from Corrientes province, Argentina (Hamann, 1992). Other known species of *Catadiscus* are associated to amphibians and reptiles, and while other adult trematodes have also been recorded in other mollusks (e.g., Martorelli, 1989), this is the only record of one inhabiting the intestine of an ampullariid snail (Hamann, 1992). No information is available about the effects of this endosymbiotic trematode on *P. canaliculata*. Its life cycle is unknown, but considering the known life cycles of Paramphistomidae, *P. canaliculata* may become infested by ingesting metacercariae found on plants and other substrata.

Symbiotic associations with Bacteria

Epibiotic bacteria:

Di Persia and Radici de Cura (1973) have noted the presence of filamentous cyanobacteria of the genera *Oscillatoria* and *Lyngbya* (Oscillatoriales) living in the epibiotic community of the periostracum of *P. canaliculata*. They also mentioned *Nostoc commune*, but only in some individual snails. The same authors mention numerous “filamentous bacteria” within the predominantly algal mat (probably chains of heterotrophic coccoid and rod-like bacteria).

Extracellular endosymbiotic bacteria:

Also, as in many other mollusks (e.g. Rosenberg and Breiter, 1969), the gut of *Pomacea canaliculata* is colonized by numerous coccoid or rod-like heterotrophic bacteria. However, we are not aware of any attempt of identifying them in *P. canaliculata*, and of exploring their possible functional implications.

In the latter respect, we have recently observed (Vega *et al.*, 2005) that *Pomacea canaliculata* can live on a cellulose-only diet for at least two months, which would indirectly indicate the presence of a cellulase in their gut, and that this cellulase may originate from the gut bacterial symbionts, as shown by Brendelberger (1997) in a pulmonate snail.

However, it should also be mentioned that evidences for endogenous animal cellulases (first shown in the termite *Reticulitermes speratus*, Watanabe *et al.*, 1998) have later been found in several species, including mollusks (e.g., *Haliotis discus hannai*, Suzuki *et al.*, 2003).

An obligate endocytobiotic bacterium:

In our previous review (Castro-Vazquez *et al.*, 2002) we suggested the existence of a symbiotic association of *P. canaliculata* to a large prokariont bearing chlorophyll-like pigment/s. The putative symbiotic corpuscles were found in midgut gland and feces of all *P. canaliculata* individuals from various places, which would indicate an obligate symbiotic association. Similar pigmented corpuscles were also found in the midgut gland and feces of *P. insularum* and *P. scalaris,* as well as in *Asolene pulchella*. Although the search for the presence of putative symbiotic elements in the latter species was not as thorough as that in *P. canaliculata*, both at the individual and population levels, it was also suggested that they would be obligate symbioses. The examination of a single individual of *Marisa cornuarietis*, another Neotropical ampullariid species, did show pigmented corpuscles in the midgut gland, but they did not appear to be eliminated in the feces.

Two pigmented corpuscular types within the cells of the midgut gland of *P. canaliculata* (identified as C and K corpuscles) appear to be morphotypes of the same organism, since transitional forms between typical C and K corpuscles can be seen in the midgut gland (Koch *et al.*, 2005). Typical C corpuscles are rounded, 14 µm width, granule-containing bodies, which are encased in an electron dense wall. Sometimes an outer membrane could be seen detached from the external wall of C corpuscles, which may also contain some inner membranes but not true thylacoids. K corpuscles, on their part, are dark brown, either oval or club shaped bodies (36 µm length, 14 µm width) which appear made of electron dense lamellae surrounding a core of coarse globules (Castro-Vazquez *et al.*, 2002; Koch *et al.*, 2005). Also, seemingly nude forms of C corpuscles, i.e., without a wall but with a double membrane surrounding them, have also been shown within the gland's alveolar cells (Castro-Vazquez *et al.*, 2002; Koch *et al.*, 2005).

The mean corpuscular DNA content were estimated for glandular C corpuscles and found to be higher than known bacterial genome sizes, which would suggest that more than one genome copy may be present in a single corpuscle (Vega *et al.*, 2005). Strong evidence for the bacterial nature of these corpuscles has come from the amplification of a 1500 bp DNA fragment corresponding to the bacterial 16S rRNA gene, using DNA extracted from glandular C and K corpuscles as template (Vega *et al.*, 2005). However, DNA sequence identification of the symbiont, as those recently obtained for the cyanobacterial endocytobionts associated to the diatoms *Climacodium frauenfeldianum* (Carpenter and Jason, 2000) and *Rhopalodia gibba* (Prechtl *et al.*, 2004) is still wanting. Nevertheless, both the size and the chlorophyll-like pigment/s of this bacterium strongly suggest that it should be a cyanobacterium in the order Chroococcales or Pleurocapsales.

It should be noted that the Cyanobacteria have been particularly successful in developing symbiotic associations during evolution, involving protists, fungi, plants and animals; however, known cyanobacterial endocytobioses are comparatively rare (Raven, 2002). In particular, cyanobacterial/animal endocytobioses have only been described in the marine sponge *Siphonocalina tabernacula* and in the didemnid ascidian *Lissoclinum punctatum* (Hirose *et al.*, 1996, 1998). In both cases, most cyanobionts were located extracellularly in the host's tissues, while only some were found within amoebocytic, presumably phagocytic cells.

Nothing is known of the functional significance of this mollusk/bacteria endocytobiosis. Even though C corpuscles have chlorophyll-like pigment/s, it should be stressed that they would not be able to perform photosynthesis in the dark environment of the ampullariid midgut gland. Instead, chloroplasts occupying the cells of the many terminal midgut gland tubules located below the slug´s transparent dorsal mantle, are able to photosynthetically reduce carbon, and to transfer photosynthate to their host (Rumpho *et al.*, 2000).

Transmission of the bacterial endocytobiont of *P. canaliculata* seems to occur vertically, i.e. it is directly transferred from mother to offspring, since pigmented corpuscles appeared in juveniles that were hatched aseptically and that were afterwards cultured in sterile media (Koch *et al.*, 2003). So far, we have been unable to culture the endocytobiont *in vitro* (Koch and Castro-Vazquez, unpublished). However, since both C and K morphotypes are eliminated in large quantities in the feces, we searched for them in the environments where *P. canaliculata* lives. Morphologically "healthy" C corpuscles have been thus observed in the mud of limnotopes inhabited by *P. canaliculata*, even at the end of winter (i.e., after many months of snail quiescence). Also, they have been found in up to three years-old sediments of aquaria formerly inhabited by snails (Koch *et al.*, 2005).

Concluding considerations

The number and diversity of endosymbiotic associations in which *P. canaliculata* is involved suggests that this metazoan organism does not establish a sharp distinction between self and non-self**.** This apparently non-stringent selectivity of *P. canaliculata* to host a variety of organisms poses several theoretical and practical questions: (1) Is this condition exclusive of this species, or is it shared with other ampullariid species? As we have mentioned above, a preference for *P. canaliculata* (as compared with *P. scalaris* and *A. pulchella*) is shown by such varied symbionts as a temnocephalid worm (*T. iheringi*), a leech (*H. ampullariae*) and a cyclopoid copepod (*O. huarpium*). (2) Which are the trophic relations of these symbionts with their host, and with the other symbionts that may coexist in the snail?. (3) *P. canaliculata* has been shown susceptible to the natural infection with a symbiont, *Angiostrongylus cantonensis* (Keawjam *et al.*, 1993), to which it is not exposed in its original range (Chao *et al.*, 1987): would then be possible that other invasive species as *P. lineata* y *P. bridgesi* were able to develop new symbiotic associations in its present range, and that some of these symbioses would become a threat to man health? (4) Alternatively, as both artificial and beneficial symbioses have been developed (particularly of cyanobacteria with plants, see Gusev *et al.*, 2002), would it be possible to utilize *P. canaliculata* as both an artificial and beneficial host?

A single obligate symbiosis (an endocytobiosis) of *P. canaliculata* with a prokariont has been described (Castro-Vazquez *et al.*, 2002; Koch *et al.*, 2005; Vega *et al.*, 2005). The question of the presumptive cyanobacterial nature of this prokaryotic endocytobiont is interesting, because known cyanobacterial endocytobioses are just a few and, so far, no one has been described in a mollusk (Vega and Castro-Vazquez, in preparation). Also, though several symbioses of mollusks with intracellular chemoautotrophic bacteria have been described (Cavanaugh, 1994; Felbeck, 1987; Shively *et al.*, 1998; Windoffer and Giere, 1997), no symbiosis is known involving the cells of the midgut gland, except for the one presented here and for the already mentioned case of "kleptoplasty". Since cyanobacteria may be the ancestors of both protistan and plant chloroplasts, it would be interesting that future studies will compare the obligate (apparently cyanobacterial) endocytobiosis of ampullariids with both the kleptoplasty of elysioid slugs, and with the primordial endocytobiosis from which chloroplasts originated.

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