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Bacterial endosymbionts in *Asellus aquaticus* (Isopoda) and *Gammarus pulex* (Amphipoda) and their contribution to digestion

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Abstract

We demonstrate for the first time the presence of bacterial endosymbionts in the midgut glands (hepatopancreas) of the freshwater detritivore *Asellus aquaticus* (Isopoda), whereas the hepatopancreas of another crustacean detritivore, *Gammarus pulex* (Amphipoda), which coexists with the former species, was devoid of such bacteria. We detected both phenol oxidase and cellulase activity in hepatopancreatic extracts from both detritivores, which suggests that both of these enzymes are produced in the midgut glands of both species. After treatment with antibiotics, both the number of hepatopancreatic bacteria and enzymatic activity were reduced in the isopod hepatopancreas, but antibiotics had no effect on enzyme activity in the amphipod hepatopancreas. Feeding on microbially inactivated leaf litter did not affect enzyme activity in hepatopancreatic extracts from *A. asellus,* but increased cellulase activity was seen in the hepatopancreas of *G. pulex.* These results (1) confirm the hypothesized enzymatic adaptation of crustacean freshwater detritivores to their food sources of terrestrial origin, (2) demonstrate that the isopod *A. aquaticus,* like its terrestrial relatives, contains endosymbiotic bacteria that contribute to digestive processes, and (3) show interspecific differences between these coexisting crustacean detritivores in terms of enzyme origin and their dependence on microbiota.

Through mediating decomposition processes, detritivores play a key role in nutrient cycling in both freshwater (Graça 2001) and terrestrial ecosystems (Schaefer 1991). Terrestrial isopods (Isopoda: Oniscidea), which feed on the leaf litter of terrestrial plants, are capable of hydrolytically digesting cellulose (Hartenstein 1964, 1982; Hassall and Jennings 1975; Kozlovskaja and Striganova 1977; Kukor and Martin 1986; Zimmer and Topp 1998*a*) and oxidatively degrading phenolic leaf litter compounds (Neuhauser and Hartenstein 1976; Zimmer and Topp 1998*b;* Zimmer 1999). It has been proposed that these digestive capabilities, being adaptive to terrestrial food sources, are brought about by bacterial symbionts of the isopods' midgut glands (hepatopancreas) (Zimmer and Topp 1998*a*,*b*; Zimmer 1999; Zimmer et al. 2001, 2002). According to the results of previous studies, hepatopancreatic symbionts may have facilitated the colonization of land by isopods. Although prototypal oniscid isopods of the genus *Ligia* (Schmalfuss 1978, 1989; Carefoot and Taylor 1995) contain hepatopancreatic bacteria, no such symbionts have been found in the marine isopod species investigated so far (Zimmer et al. 2001, 2002; Wang et al. unpubl. data).

It is important to distinguish between endogenous enzymes produced in the hepatopancreas and ingested enzymes that reach the hindgut along with leaf litter-colonizing microbiota and remain active during the gut passage. Cellulases and phenol oxidases that are produced by bacterial symbionts in the hepatopancreas of terrestrial isopods are considered to be ''functionally endogenous'' (Zimmer and Topp 1998*a*). Because of an effective filter system that is present in isopods and amphipods (and most other malacostracan crustaceans) (Martin 1964; Scheloske 1976; Oshel and Steele 1988; Wägele 1989), no ingested food particles or microbiota can enter the hepatopancreas. Diverse enzymes contribute to digestive processes in the hindgut lumen, but, if a particular enzyme is not present in the hepatopancreas, it will have to be considered to be of nonendogenous origin. In this case, microbiota ingested along with the food have to be considered a source of this particular enzyme.

Detritivorous freshwater crustaceans exploit the same food source as most terrestrial detritivores—namely, the shed leaves of deciduous trees that fall into woodland creeks and ponds (e.g., Wetzel 1995). Thus, they face similar nutritional constraints by feeding on a food source that is poor in nutrients but rich in deterrent and recalcitrant compounds, such as phenolics and cellulose. Although water-soluble phenolics are readily removed from leaf litter in freshwaters through leaching, high-molecular-weight phenolics such as lignin remain present. Even though lignin is unlikely to be excessively degraded by detritivores, its partial degradation through phenol oxidation is required to gain access to the cellulose moiety of lignocellulose (e.g., Breznak and Brune 1994).

In temperate lentic freshwaters, *Asellus aquaticus* (Isopoda: Asellota) is one of the most common detritivorous crustaceans. It is frequently accompanied by *Gammarus pulex* (Amphipoda: Gammaridea), which often prefers lotic freshwaters. Although both are detritivores feeding on leaf litter, these potentially sympatric crustaceans appear to avoid competition through species-specific utilization of the avail-

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able food source (Graça et al. 1993*a*,*b*, 1994). Thus, we can assume interspecific differences with respect to digestive strategies in these coexisting crustacean detritivores. To our knowledge, no one has attempted to prove the presence of bacterial symbionts in freshwater crustaceans that might contribute to digestive processes, and relatively little is known in general about the ability of these species to digest leaf litter (but see Bjarnov 1972; Monk 1977; Bärlocher 1982; Chamier and Willoughby 1986; McGrath and Matthews 2000).

In the present study, we test three hypotheses: (1) aquatic crustacean detritivores (*A. aquaticus* and *G. pulex*) are adapted to their terrestrial food source in that they produce enzymes (cellulases and phenol oxidases) needed to digest leaf litter compounds; (2) as an adaptation to their terrestrial food source, *A. aquaticus* and *G. pulex* possess bacterial endosymbionts in their hepatopancreases that contribute to the production of these functionally endogenous enzymes; and (3) these coexisting crustacean detritivores differ in their digestive capabilities and their dependence on microbial enzymes with respect to the digestion of phenolics and cellulose.

Materials and methods

General remarks—The design of the experimental investigation reflects two important features of the functional anatomy of terrestrial and aquatic isopods. First, the hepatopancreas is the seat of digestive enzyme production; second, both particulate matter and bacteria are prevented from entering the lumen of the hepatopancreas by a structural filtering system that allows the inflow of fluids but not particles (Zimmer 2002). This has three important consequences. Ingested microbiota cannot enter the hepatopancreas, so bacteria located in this organ are likely to be endosymbionts; ingested bacteria can enter the hindgut region and thus may contribute to the enzyme profile of the host; and enzymes that occur in the hindgut but not the hepatopancreas will be of exogenous origin, whereas functionally endogenous enzymes will be present in both the hepatopancreas and the hindgut.

Experimental design—Both *A. aquaticus* and *G. pulex* were collected in freshwaters in the vicinity of Kiel, Germany. In the laboratory, isopods and amphipods were kept separately in aerated tap water at 12°C and fed with mixed leaf litter taken from the field for up to 7 d before they were used in the experiment.

For the quantification of bacterial hepatopancreatic symbionts, midgut glands (hepatopancreas) were extracted from the body cavity with flame-sterilized forceps, separated from the foregut and hindgut, and immediately homogenized in 1,000 μl of autoclaved double-distilled water. Samples were diluted in 1,000 μl of a 4% formaldehyde solution and subsequently stained with 4′6′-diamidino-2-phenyl-indole (DAPI) for direct bacterial counts under an epifluorescence microscope. For the reduction of bacterial numbers, a commercially available mixture of antibiotics (penicillin G, streptomycin sulfate, and amphotericin B; Sigma) was applied by adding 100 mg to 100 ml of aerated tap water, which resulted in a final concentration of 1,000 units of penicillin G ml^{-1}, 1 mg of streptomycin sulfate ml^{-1}, and 2.5 μg amphotericin ml^{-1}. Isopods and amphipods were maintained in this antibiotic solution for 7 d at 12°C. This kind of application of antibiotics was chosen to affect leaf litter microbiota (both bacteria and fungi) directly as well as hepatopancreatic bacteria through ingested water that, in contrast to food particles (see above), passes into the hepatopancreas. As for terrestrial isopods (Zimmer and Topp 1998*a*,*b*; Zimmer 1999), these antibiotics had no long-term effects on consumption or the duration of the molting cycle in *A. aquaticus* or *G. pulex* (Zimmer unpubl. data).

In an additional experiment, we fed isopods and amphipods with microbially inactivated leaf litter for 7 d, to reduce the number and activity of ingested microbiota without affecting the number and activity of hepatopancreatic bacteria (Zimmer 1999). Leaf litter was air-dried; subsequently, single leaves were irradiated with ultraviolet (UV) C radiation from both sides for 12 h each. After an incubation in autoclaved pond water for 3 d (to activate propagules that survived the UV treatment), wet leaves were irradiated and then air-dried again. Essentially no enzymatic activity was detectable on inactivated leaf litter. During feeding on inactivated leaf litter, animals were kept in autoclaved pond water at 12°C; leaf litter and water were replaced daily.

With respect to digestive enzymes, we distinguished between those that can be considered functionally endogenous, because they are present in the hepatopancreas (and the hindgut, see above), and those that have been ingested along with food material and are not present in the hepatopancreas but are only present in the hindgut. For the determination of cellulase activity (according to Skambracks and Zimmer 1998, with an incubation of crystalline cellulose for 18 h at 12°C) or the extent of phenol oxidation (as described by Zimmer and Topp 1998*b*, using 50 mmol L^{-1} catechin in 20% ethanol as the phenolic substrate), midgut glands were extracted from the body cavity and separated from the fore- and hindgut. Both the hepatopancreas and hindgut were immediately homogenized separately at 4°C in 500 μl of autoclaved phosphate buffer (pH 5.5). These suspensions were directly used for the determination of enzyme activity (see above). In all cases, tissue-free assays served as controls. Furthermore, boiling the samples prior to the addition of the phenolic substrate served as control for nonenzymatic phenolic autoxidation in phenol oxidase assays.

By separating midgut glands from the fore- and hindgut, we were able to unambiguously distinguish between (1) functionally endogenous enzymes produced in the hepatopancreas and enzymes ingested along with the leaf litter and (2) truly endosymbiotic bacteria present in the hepatopancreas and microbiota ingested along with the leaf litter. The application of antibiotics allowed for the detection of a relationship between bacterial numbers in the hepatopancreas and the activity of functionally endogenous enzymes. Offering microbially inactivated leaf litter as food allowed for the distinction between enzymes ingested along with the food (that might have passed the filter that prevents particles from entering the hepatopancreas) and those produced in the hepatopancreas.

Table 1. Characteristics of midgut glands (hepatopancreas) of the crustacean detritivores, *A. aquaticus* and *G. pulex,* after feeding on a natural mix of leaf litter (''field''), after antibiotic treatment (''antib.''), and after feeding on microbially inactivated leaf litter (''inact.''). Data are given as mean ± standard deviation ($n = 10$).

	Bacteria (cells per hepatopancreas)			Cellulase activity (μg glucose h^{-1} per hepatopancreas)			Phenol oxidation (ΔA_{340} min^{-1} per hepatopancreas)		
Treatment	field	antib.	inact.	field	antib.	inact.	field	antib.	inact.
A. aquaticus	$(1\pm0.5)\times10^6$	$(3\pm1)\times10^5$	$(9\pm6)\times10^5$	4±1	1±1	5±3	0.003±0.001	0.0016±0.0009	0.005±0.003
G. pulex	0	0	0	8±4	6±2	12±5	0.016±0.007	0.0013±0.0007	0.012±0.006

Results

Bacterial symbionts—DAPI staining revealed direct microscopic counts of $(1 \pm 0.5) \times 10^6$ (mean ± SD; $n = 10$) bacteria in the hepatopancreas of *A. aquaticus* (Table 1). Antibiotic treatment significantly reduced these bacterial symbionts by ~67%, to $(3 \pm 1) \times 10^5$ cells per hepatopancreas (Mann-Whitney *U*-test; $U = 5.0$; $P < 0.001$; $n = 10$). Although feeding on inactivated leaf litter did not affect bacterial numbers in the hepatopancreas of *A. aquaticus* ($P > 0.4$), the hindgut was significantly less densely colonized after 1 week's feeding on this litter ($P < 0.01$; data not shown). In contrast to our findings in *A. aquaticus,* we did not find bacteria in the hepatopancreas of *G. pulex.*

Cellulase activity—Both detritivores exhibited considerable cellulase activity in their hepatopancreases (Table 1). In *A. aquaticus* midgut glands, 4 ± 1 μg of glucose per hour was released from cellulose ($n = 10$). Similar values (3 ± 2 μg of glucose per h^{-1}) were obtained from hindgut extracts. Cellulose was essentially not hydrolyzed in hepatopancreas extracts of antibiotic-treated isopods (Table 1), but cellulase activity in the hepatopancreas was not affected by feeding on inactivated leaf litter ($P > 0.8$). Similarly, although antibiotic treatment significantly reduced cellulase activity in the hindgut ($P < 0.05$), cellulase activity in the hindgut even slightly (albeit insignificantly: $P < 0.1$) increased after feeding on inactivated litter (data not shown).

As is obvious from Table 1, cellulose hydrolysis in hepatopancreatic extracts occurred at even higher rates in *G. pulex* than in *A. asellus.* By contrast, hindgut extracts of *G. pulex* exhibited comparably low cellulase activities of 4 ± 2 μg glucose h^{-1}. Antibiotics did not significantly reduce cellulase activity in the hepatopancreas of *G. pulex* ($P > 0.3$; Table 1), nor did they affect cellulase activity in the hindgut ($P > 0.6$; data not shown). Feeding on inactivated leaf litter slightly increased cellulase activity in both the hepatopancreas (Table 1) and the hindgut (data not shown) of *G. pulex.*

Antibiotic treatment significantly affected hepatopancreatic cellulase activity in *A. aquaticus,* which indicates an involvement of hepatopancreatic bacteria in cellulose hydrolysis but not in *G. pulex* (analysis of variance [ANOVA]; Table 2). Feeding on inactivated leaf litter had no effect on cellulase activity in the hepatopancreas of *A. asellus* but enhanced cellulase activity in *G. pulex* (ANOVA; Table 2).

Phenol oxidation—Hepatopancreatic extracts from both *A. aquaticus* and *G. pulex* readily oxidized catechin (Table

Table 2. ANOVA tables for the influence of antibiotics and inactivation of leaf litter on enzymatic activity in the midgut glands of the crustacean detritivores *A. aquaticus* and *G. pulex.* df, degrees of freedom; SS, sum of squares; F, test statistic; *P*, probability.

	Cellulase activity				Phenol oxidation		
Treatment	df	SS	F	*P*	SS	F	*P*
A. aquaticus							
Antibiotics	1	0.0003	9.52	0.006	0.00001	10.05	0.005
Error	18	0.0006			0.00002		
Total	19	0.0009			0.00003		
G. pulex							
Antibiotics	1	0.000001	0.01	0.941	0.000001	1.86	0.189
Error	18	0.002615			0.000003		
Total	19	0.002616			0.000004		
A. aquaticus							
Inactivation	1	0.00076	0.36	0.556	0.000001	0.01	0.916
Error	18	0.003809			0.000002		
Total	19	0.004569			0.000003		
G. pulex							
Inactivation	1	0.1398	6.51	0.021	0.000001	3.19	0.112
Error	18	0.3864			0.000003		
Total	19	0.5262			0.000004		

1). Because there was essentially no phenol oxidation in tissue-free control assays and in boiled assays (data not shown), we can assume phenol oxidation to be due to enzymatic activity rather than phenolic autoxidation. Antibiotics significantly reduced the activity of phenol oxidases in the *A. aquaticus* hepatopancreas ($P < 0.05$; Table 1) and hindgut ($P < 0.05$; data not shown). By contrast, phenol oxidation was not affected by feeding on inactivated leaf litter in both the hepatopancreas ($P > 0.9$) and hindgut ($P > 0.4$; data not shown) of *A. asellus.* In *G. pulex,* neither antibiotics nor feeding on inactivated leaf litter affected phenol oxidation in the hindgut ($P > 0.1$; data not shown) or the hepatopancreas ($P > 0.2$; Table 1).

Antibiotic treatment significantly affected the activity of hepatopancreatic phenol oxidases in *A. aquaticus,* which indicates an involvement of hepatopancreatic bacteria in phenol oxidation that was not seen in *G. pulex* (ANOVA; Table 2). Feeding on inactivated leaf litter had no effect on phenol oxidation in the hepatopancreas of *A. asellus* or *G. pulex* (ANOVA; Table 2).

Discussion

General remarks—Leaf litter, the food source of many terrestrial and freshwater detritivores, is characterized by a low nutrient content and a high content of deterrent and recalcitrant compounds such as phenolics and cellulose. Although many phenolics are prone to leaching in aquatic environments, cellulose remains bound to lignin, a recalcitrant polymer of phenol derivates, as lignocellulose, and the degradation of cellulose requires the partial degradation of lignin. Thus, detritivores are in need of degrading cellulose and diverse phenolic compounds (e.g., Breznak and Brune 1994). In terrestrial isopods, these digestive characteristics are apparently brought about by bacterial symbionts that are present in high numbers in the hepatopancreas (Zimmer and Topp 1998*a,b*; Zimmer 1999; Zimmer et al. 2001, 2002). Thus, we conclude that these symbionts facilitate the use of leaf litter as food source.

With respect to digestive enzymes in crustaceans, we must distinguish between those that can be considered to be functionally endogenous and those that have been ingested along with food material (see first section). If a particular enzyme is present in the hindgut but not in the hepatopancreas, we will consider it to have been ingested. If, however, a particular enzyme is present in the hepatopancreas (i.e., it will also be present in the hindgut), we will consider it to be either of endogenous origin or produced by hepatopancreatic endosymbionts (functionally endogenous), although we cannot completely rule out the possibility that ingested enzymes enter the hepatopancreas. Similarly, we must distinguish between truly endosymbiotic bacteria inside the hepatopancreas lumen (as in terrestrial isopods; Zimmer and Topp 1998*a*; Zimmer et al. 2001, 2002) and those microbiota that reach the hindgut lumen along with the food but cannot enter the hepatopancreas because of an effective filter preventing particles from entering the midgut glands (Martin 1964; Scheloske 1976; Oshel and Steele 1988; Wägele 1989). Thus, if we find bacteria inside the hepatopancreas, we will consider them to be endosymbiotic.

Hepatopancreatic bacteria and enzymes—As the present results indicate, there are no such bacteria in *G. pulex.* By coincidence, neither bacteria nor bacterial DNA could be detected by means of molecular biology in this amphipod (Wang et al. unpubl. data). However, we detected both phenol-oxidizing and cellulolytic activity in the hepatopancreas. It is unlikely that these activities derived from microbial enzymes ingested along with the food that might have passed the hepatopancreatic filter, because the microbial inactivation of leaf litter did not affect phenol oxidation, either in the hindgut or in the hepatopancreas, and it even increased cellulase activity in the hepatopancreas. Thus, because of the lack in hepatopancreatic endosymbionts, the cellulases and phenol oxidases appear to be of endogenous origin in *G. pulex.* The increase in hepatopancreatic cellulase activity in *G. pulex* that were fed inactivated litter may be due to a feedback response to low cellulase activity in the food, but further detailed studies are needed to decide upon this issue.

In contrast to *G. pulex,* hepatopancreatic bacterial symbionts that are significantly reduced by ingested antibiotics are present in *A. aquaticus.* As in *G. pulex,* both cellulases and phenol oxidases were active in the hepatopancreas of *A. asellus.* Along with the reduction of bacterial numbers in the hepatopancreas, antibiotics reduced the activity of both cellulases and phenol oxidases in hepatopancreatic (and hindgut) extracts. Hence, we propose that these enzymes are produced by endosymbiotic bacteria in the hepatopancreas of *A. aquaticus.* The same conclusion has been drawn from similar experiments with terrestrial isopods (Zimmer and Topp 1998*a,b*; Zimmer 1999) that feed on the same food source, terrestrial leaf litter, and with coastal isopods feeding on stranded seaweed and diatoms (Zimmer et al. 2001, 2002), although we cannot definitely rule out that these enzymes are produced by the isopod itself (for a discussion of this topic in terrestrial isopods, see Zimmer 2002), with antibiotics affecting both hepatopancreatic bacteria and endogenous enzyme production independently.

Obviously as an adaptation to their terrestrial food source, the freshwater detritivores *A. aquaticus* and *G. pulex* exhibit (functionally) endogenous cellulases. Slightly higher rates of cellulose hydrolysis (10–20 μg glucose mg^{-1} h^{-1}) have been determined in the hepatopancreas of a terrestrial isopod, *Porcellio scaber,* and have been proposed to be of endosymbiotic origin (Zimmer and Topp 1998*a*). According to the obvious lack of symbiotic bacteria in the hepatopancreas, *G. pulex* appears to produce cellulases endogenously (see above). McGrath and Matthews (2000) demonstrated endogenous cellulase activity in *G. lacustris* but only found endoglucanase active toward soluble carboxymethylcellulose, whereas they assumed cellobiohydrolases, which are needed to degrade native crystalline cellulose, to be acquired from exogenous microbial sources. Similar conclusions have been drawn from studies on a variety of invertebrates (Martin 1987, 1991 and references therein), but earlier studies reported contrasting results. Monk (1976) found both cellobiohydrolase and endoglucanase activity in *G. pulex* and *G. lacustris* and later concluded that these enzymes are of endogenous origin (Monk 1977). By contrast, Bärlocher (1982) proposed a significant contribution of ingested fungal enzymes to cellulose digestion in *G. fossarum* and corroborated

this conclusion in a later study on *G. tigrinus* (Bärocher and Porter 1986). In that same year, Chamier and Willoughby (1986) came to the conclusion that fungal enzymes contribute little to digestion in *G. pulex*, producing both cellobiohydrolase and endoglucanase endogenously. Here, we present evidence for endogenous activity toward native cellulose in *G. pulex* (see above); thus, this detritivore does not seem to depend on microbial activity for the degradation of the most important compound of its food source. By contrast, *A. asellus* appears to rely on functionally endogenous cellulases of endosymbiotic origin. While thoroughly studying hepatopancreatic amylases in *A. asellus,* Robsen (1979), however, found no evidence for cellulolytic activity in the hepatopancreas of *A. asellus.*

Similar to the present evidence for cellulases of endosymbiotic origin, hepatopancreatic bacteria in *A. aquaticus* appear to provide phenol oxidases, too. By contrast, *G. pulex* obviously produces digestive phenol oxidases endogenously (see above). Phenol oxidases are common in the hemolymph and the cuticle of arthropods, where they are involved in the immune response (e.g., Gillespie et al. 1997) and sklerotization (e.g., Anderson 1985), respectively. These enzymes, however, are not produced by digestive glands, nor are they assumed to be involved in digestive processes inside the gut lumen.

In summary, the coexisting freshwater crustacean detritivores *A. aquaticus* and *G. pulex* are enzymatically adapted to their terrestrial food source, but they appear to have evolved different ways of utilizing it, confirming our hypotheses (1) and (3) that predicted cellulases and/or phenol oxidases of (functionally) endogenous origin and species-specific ways of utilizing their terrestrial food source, respectively. Although the isopod *A. aquaticus,* like its terrestrial relatives, appears to have acquired bacterial endosymbionts that provide essential digestive enzymes (hypothesis 2), the amphipod *G. pulex* apparently produces both cellulase and phenol oxidases in its hepatopancreas. Both strategies allow the use of terrestrial leaf litter as a food source. It is, however, interesting to speculate on reasons why amphipods were capable of the evolutionary invention of endogenous cellulases, whereas isopods were not (but see Ray and Julian 1952 for *Limnoria tripunctata*). Both are probably capable of producing the phenol oxidases that are involved in the immune response and molting (see above) endogenously, but these enzymes usually do not participate in digestive processes. Because, on the other hand, we may argue that the possibly expensive production of endogenous cellulases is not necessary for an animal that harbors endosymbionts that provide functionally endogenous cellulases, it is also interesting to speculate on reasons for why amphipods were apparently unable to acquire hepatopancreatic bacteria. This topic is currently under investigation in our laboratory in the context of studies on host-symbiont coevolution in isopods and their hepatopancreatic bacteria.

In the same context, the existence of endosymbiotic bacteria in Asellota may be of significance with respect to our understanding of isopod phylogeny. Like Oniscidea, freshwater Asellota probably have marine ancestors (Wägele 1989); the phylogenetic relation of these two taxa, however, has been discussed controversially (Schmalfuss 1989; Wägele 1989; Brusca and Wilson 1991). Because marine relatives of both Oniscidea and Asellota do not contain hepatopancreatic symbionts that contribute to digestion (Zimmer et al. 2001, 2002; Wang et al. unpubl. data), we may speculate on a role of these bacteria in the evolutionary shift to using terrestrial food sources (Zimmer 2002). Such bacterial symbionts in the hepatopancreas of *A. aquaticus* would either hint on a close phylogenetic relationship of these taxa—that is, on a common marine ancestor that harbored endosymbiotic bacteria in its hepatopancreas—or might just be a convergent adaptation of freshwater Asellota and terrestrial Oniscidea to feeding on leaf litter. The elucidation of this issue requires details about the phylogenetic relationship of endosymbionts in oniscid and asellote isopods; molecular-biological studies on this topic are in progress in our laboratory.

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