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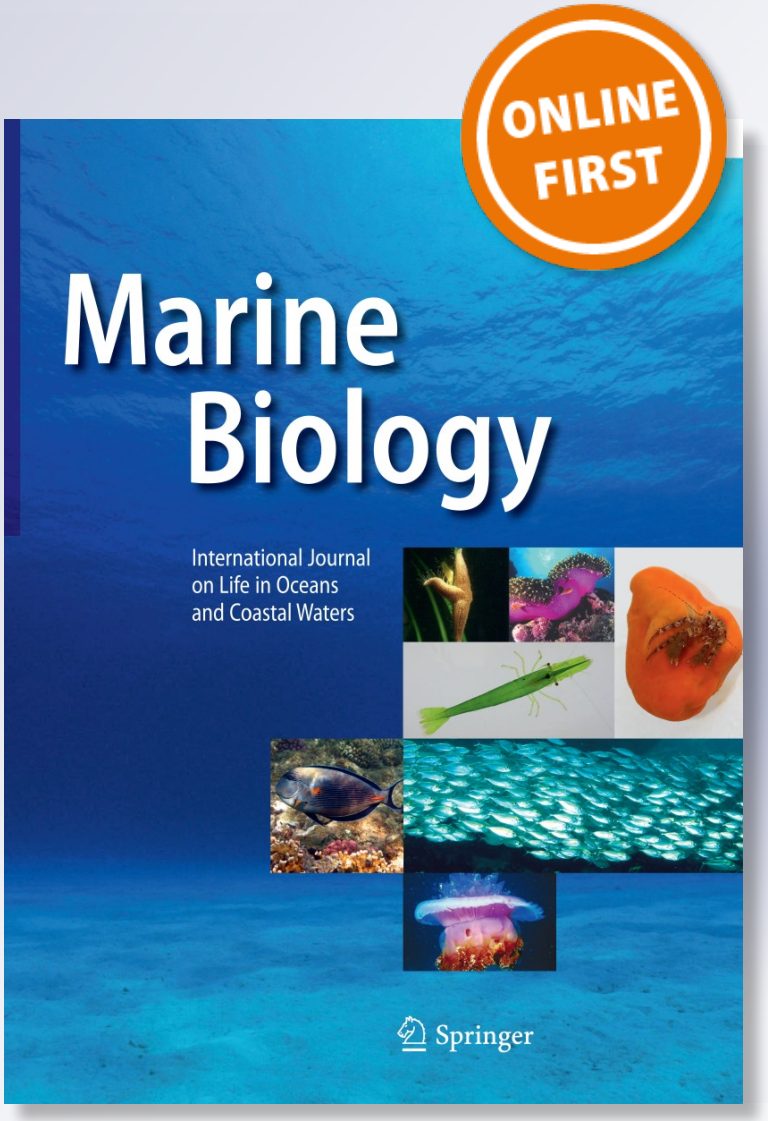

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ORIGINAL PAPER

Bacterial diversity of the sediments transiting through the gut of *Holothuria scabra* (Holothuroidea; Echinodermata)

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Abstract This work analyzes bacterial diversity of sediments transiting through the gut of *Holothuria scabra* which is an important bioturbator in tropical shallow waters. This edible holothurian species has a social and economic importance for coastal populations in many developing countries. Bacterial biodiversity was analyzed by sequencing the 16S rRNA of bacterial cultures and clones. DAPI and FISH methods were used to determine and compare the number of bacteria found in the various gut compartments. A total of 116 phylotypes belonging to the γ-Proteobacteria (60.5 %), α-Proteobacteria (24.5 %), Bacteroidetes (6 %), Actinobacteria (2.75 %), Fusobacteria (1.75 %), Firmicutes (1.75 %), Cyanobacteria (1.75 %) and δ-Proteobacteria (1 %) were identified. The number of bacteria is significantly greater (1.5×) in the foregut than in the ambient sediments. The number of bacteria significantly decreases in the midgut and remains stable until defecation. Some γ-Proteobacteria, especially *Vibrio*, are less affected by digestion than other bacterial taxa. The season has an impact on the bacterial diversity found in the sediments transiting through the gut: in the dry season, γ-Proteobacteria are the most abundant taxon, while α-Proteobacteria dominate in the rainy season. *Vibrio* is the most frequent genus with some well-known opportunistic pathogens like *V. harveyi, V. alginolyticus* and *V. proteolyticus*. Findings show that sediment-associated microbial communities are significantly modified by *H. scabra* during their transit through the gut which supports the view that holothurians play a substantial role in the structuring of bacterial communities at the sediment–seawater interface.

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Introduction

Holothurians, or sea cucumbers, form a class of echinoderms of about 1,400 species that are mainly deposit feeders. Their juveniles are important preys in food webs (So et al. 2010), and their adults are among the most efficient bioturbators in many ecosystems (Birkeland 1988; Uthicke 2001; Wolkenhauer et al. 2010). Many aspidochirote holothurians feed on large quantities of sediment and convert organic matter into animal tissue and nitrogenous wastes, these wastes being used by photoautotrophs such as macroalgae and diatoms (Uthicke and Klumpp 1998; Uthicke 2001). In addition, through bioturbation, the burying holothurians oxygenate sediments (Bakus 1973; Massin 1982; Uthicke 1999, 2001; Purcell 2004; Mangion et al. 2004) and increase the productivity of sea grasses (Wolkenhauer et al. 2010). In some ecosystems, the

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removal of holothurians is presumed to reduce primary production of the whole food chain (Purcell et al. 2013). Holothurians also process carbonate sand and rubble through their digestive tract and dissolve $CaCO_3$ as part of their digestive processes (Schneider et al. 2011). In a healthy reef, the dissolution of carbonate sediments due to holothurian activity is an important component of the natural $CaCO_3$ turnover and a substantial source of alkalinity (Schneider et al. 2011).

Apart from their important ecological role, holothurians have a social and economic importance for several coastal populations in developing countries (Purcell et al. 2013). Today, sea cucumber aquaculture exists at the industrial or semi-industrial level for three species: the East Pacific tropical *Isostichopus fuscus,* the temperate Sino-Japanese *Apostichopus japonicus* and the tropical Indo-West Pacific *H. scabra*. *H. scabra* is the most promising candidate for many regions of the Indo-West Pacific because it has a high value on the Chinese market and because cultured juveniles can be reared in marine pens kept by coastal villagers (Robinson and Pascal 2009). Sea cucumber farming is currently done in Madagascar (Eeckhaut et al. 2009; Robinson and Pascal 2009, 2012; Eriksson et al. 2012) and in some other countries like Australia, Vietnam and Philippines (Purcell et al. 2013).

Bacteria have been observed in the gut of various holothurians (Féral 1980; Odintsov 1981), especially in deep-sea species (Khripounoff and Sibuet 1980; Deming et al. 1981; Deming and Colwell 1982; Dilmore and Hood 1986; Amaro et al. 2012), and it is largely accepted that they are important components assimilated by holothurians (Yingst 1976). Despite the ecological and commercial importance of holothurians, their bacterial flora has only been studied in detail in the tropical shallow-water species *Holothuria atra* (Ward-Rainey et al. 1996). The latter investigators found that *Vibrio* was the most abundant taxon; other isolates included members of the genus *Bacillus*, the α-Proteobacteria and γ-Proteobacteria and the *Cytophaga–Flavobacterium–Bacteroides* lineage.

The aim of the present study was to analyze the bacterial community found in the sediments before and during their transit through the digestive tract of *H. scabra*. The microhabitat of *H. scabra* differs from that of *H. atra* as the first is found in sea grass beds (Hamel et al. 2001). Adults of *H. scabra* feed on sediments during the night and burrow during the day (Hamel et al. 2001). We analyzed the bacterial composition of (1) sediment from the natural habitat of *H. scabra*, (2) the surrounding seawater and (3) sediment in the three segments that compose its digestive tract. We also compared the bacterial composition of ambient and ingested sediment during the dry and rainy seasons.

Materials and methods

Sampling

Live adults of *H. scabra* were obtained from "Madagascar Holothurie" based in Toliara (Eeckhaut et al. 2009). Larvae resulting from in vitro fertilization (Léonet et al. 2009) were raised for 3 weeks in hatchery (Eeckhaut et al. 2009). After metamorphosis, epibenthic juveniles of *H. scabra* were isolated and kept in tanks with sediments containing *Thalassia* and *Thalassodendron* sea grasses until they reached about 3 cm in length. At the end of this stage, epibenthic individuals developed a burrowing behaviour and became endobenthic (Lavitra et al. 2010). They were transferred into closed ponds of 32 m^2 containing sediment coming from the natural habitat of adults. *H. scabra* juveniles stayed in these ponds until they reached a refuge length of 6 cm. They were then transferred into sea pens of 600 m^2 located in the intertidal zone where sea grass beds abound (Lavitra et al. 2010). Epibenthic juveniles (3 cm long), endobenthic juveniles (6 cm long) and adults (22 cm long) were used in the study. Samples were obtained in 2008 during the dry season and in 2009 during the rainy season (see below). All individuals were dissected under a binocular microscope.

Bacterial cultures and identification of isolates

Heterotrophic aerobic bacteria from seawater, from the sediment where *H. scabra* were found (i.e., first centimetre of the substrate sampled randomly), from the three gut segments and from the faeces were isolated on solid LB medium. The medium was composed of tryptone (10 g L^{-1}), yeast extract (5 g L^{-1}), agar (15 g L^{-1}) and NaCl (30 g L^{-1}) and was made with MilliQ water. Seawater ($n = 10$), in situ sediments ($n = 10$) and faeces ($n = 10$) were swabbed with a sterile cotton that was then used to inoculate culture plates. Individuals of each type [epibenthic juveniles of 2 cm long ($n = 10$), endobenthic juveniles of 6 cm long ($n = 10$) and endobenthic adults of 22 cm long ($n = 10$)] were dissected using sterile instruments. The sediments inside gut segments were swabbed and bacteria were streaked on agar plates. After 24 h of incubation at 28 °C, colonies were isolated and streaked two more times on new agar plates to insure purity. Isolated colonies were preserved in absolute ethanol (100 %) and the bacterial phylotypes characterized by DNA sequencing. Bacterial DNA from 5 to 10 mg subsamples was extracted using an Invisorb spin tissues minikit (Invitek). A 550-bp-long 16S rRNA gene fragment was amplified by touchdown-PCR using the PuReTaq Ready-To-Go PCR beads kit (GE Healthcare) and a Thermal iCycler (Bio-Rad). The PCR primers used were the

DS907R (5′-CCGTCAATTCCTTRAGTTT-3′) and the GM5F (5′-CCTACGGGAGGCAGCAG-3′) of Teske et al. (1996). The PCR cycle included a denaturation step of 30 s at 95 °C, annealing of 30 s (the annealing temperature was decreased by 0.5 °C every cycle during 22 cycles, from 64 to 53 °C) and elongation of 30 s at 72 °C. Thirteen cycles were then performed at an annealing temperature of 53 °C. A final elongation step of 7 min at 72 °C was performed. PCR products were visualized on 1 % agarose gels stained with ethidium bromide (0.5 mg L^{-1}) using a GelDoc (Bio-Rad) and the Quantity One 4.1 software. The amplified products were purified with a QIAQuick Purification kit (Qiagen) and sequenced with BigDye Terminator v1.1 Cycle Sequencing Kit (ABI) with GM5F and DS907R primers. Cycle sequencing included a denaturation step of 1 min at 96 °C and then 30 cycles including a 1-min denaturation at 96 °C, a 5-s annealing step at 55 °C and a 4-min elongation step at 60 °C. Sequences were obtained on an ABI Prism 3100 genetic analyzer.

Cloning of the environmental 16S rRNA

Ten clone libraries were created: (1) 2 for seawater, (2) 2 for the sediment from the substrate and (3) 6 (2 × 3) for the sediments extracted from segments 1, 2 and 3 of the gut. The number of clones obtained from libraries varied from 32 to 55. Samples were obtained in June 2008 (dry season) and January 2009 (rainy season). Two adults were used for each season, as well as two samples of seawater and substrate. All samples were preserved in 100 % ethanol, and DNA was extracted as described above. DNA from the complete 16S rRNA gene was amplified with primers 8F and 1492R of Buchholz-Cleven et al. (1997) using 3 µL of purified DNA. For the PCR, a 5-min initial denaturation step (95 °C) was used followed by 30 cycles (denaturation, 1 min at 94 °C; annealing, 1 min at 50 °C; elongation, 1 min at 72 °C). A final elongation at 72 °C was performed for 10 min. PCR products were purified with a QIAQuick Purification kit (Qiagen) and cloned with a TOPO TA cloning Kit (Invitrogen). Clones containing the complete 16S rRNA gene were identified in libraries, selected (using vector primers M13) and their plasmids isolated with a QIAprep miniprep kit (Qiagen). Sequences between 400 and 950 bp were obtained using an ABI Prism 3,100 genetic analyzer and the primer GM5F.

Total bacterial count

Total bacterial counts were evaluated in the sediments by DAPI staining. Samples ($n = 4$ for each type of sample) from the sediment of the substrate, from the faeces and from the sediments in the foregut, midgut and hindgut were fixed in 4 % paraformaldehyde, rinsed three times with 0.2 µm filtered seawater and stored at −20 °C in a 1/1 mix of 0.2 µm filtered seawater and 100 % ethanol. A fraction of each sample (3 g of ww) was placed in a tube and 750 µL of the previous mix was added. The sediment suspensions were sonicated three times during 30 s using the pulse mode of an Ika sonicator (Ika, Labortechnik, Germany) (50 W, cycle 0.5 and amplitude 80 %). Sediments were left untreated during 30 s between two runs and the probe was sterilized with ethanol. This treatment detached bacteria from the particles (Epstein and Rossel 1995; Gillan et al. 2005). After sonication, samples were left untreated for 30 s to sediment large particles. A volume of 75 µL of the supernatant was placed in a tube containing 10 mL of filtered seawater (pore size, 0.2 µm). Bacteria in the suspension were collected on an isopore membrane filter (0.2 µm, Millipore GTTP) placed over a 0.45-µm filter (Millipore HAWP). Filters were stained with DAPI (3 min. in 1 µg mL^{-1}), rinsed with MilliQ water and 70 % ethanol, air dried and mounted in Vectashield (Vector Lab., Burlingame, USA). Pictures of every filter were taken with a Carl Zeiss Axio Scope A1 microscope fitted for epifluorescence microscopy. Pictures were obtained with a Carl Zeiss AxioCam Icc3 camera controlled with Carl Zeiss AxioVision software. In each filter, 14 pictures were taken randomly along two transects, a vertical and a horizontal one, that crossed on the centre of the filter at right angles (Austin 1989). The bacteria in each picture were manually pointed and counted with the Jeol SamAfore 3.0 pro software. Such a counting scheme guarantees the lowest amount of errors (Kirchman et al. 1982, Montagna 1982). The number of bacteria in 14 pictures was multiplied by 1468 in order to obtain the number of bacteria in a filter and again multiplied by 10 to obtain the number of bacteria in the supernatant. The total number of bacteria was expressed by g (dw) of sediment.

Fluorescent in situ hybridization (FISH)

For FISH, samples ($n = 4$ for each type of sample) from sediment of the substrate and from the faeces were fixed, treated and mounted as described above. Five oligonucleotide probes were used: EUB338 for Bacteria (Amann et al. 1990), CF319a for the Cytophaga–Flexibacter–Bacteroides (CFB) bacteria (Manz et al. 1996), DSS658 for δ-Proteobacteria of *Desulfosarcina-Desulfococcus* group (Manz et al. 1998) and GAM42a for γ-Proteobacteria (Manz et al. 1992). These taxa are the most common in marine sediments (e.g., Teske et al. 1996; Gillan et al. 2005; Gillan and Pernet 2007). Probe NON338 was used as negative control (Wallner et al. 1993). Filter sections were placed in 0.2 mL tubes (one filter section per tube) with 150 µL of hybridization solution (0.9 M NaCl; 20 mM Tris–HCl, pH 7.5; 750 ng of probe; formamide, 10 % for

EUB338 and NON338 probes, 35 % for GAM42a and CF319a probes and finally 60 % for the DSS658 probe). The competitor probe cBET42a was used for hybridization with the GAM42a probe. For hybridization, tubes were incubated in a water bath at 46 °C for 1.5 h. The probes were purchased from QIAGEN (high-performance liquid chromatography-purified oligonucleotides labelled with Cy3 at the 5′ end). Filters were placed in a washing solution during 2 min at 48 °C (20 mM Tris–HCl, pH 7.5; 0.01 % SDS; NaCl, 450 mM for EUB338 and NON338 probes, 70 mM for GAM42a and CF319a probes, 4 mM for DSS658 probe and EDTA, 5 mM without EDTA for probes EUB338 and NON338). Filters were finally rinsed with MilliQ water, air dried, DAPI stained, placed under an epifluorescence microscope and photographed as described previously. The signal obtained with probe NON338 at each sampling site was subtracted from all the counts (Gillan et al. 2005).

Sequence analysis and statistics

PCR-generated chimeric sequences were detected and eliminated using Bellerophon (Huber et al. 2004) and DECHIPER (Wright et al. 2012) softwares. Sequences (forward and reverse) obtained from bacteria were submitted to a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) in order to identify the closest species. Sequences were aligned with MUSCLE (Multiple Sequence Comparison by Log-Expectation; http://www.ebi.ac.uk/Tools/msa/muscle/), and bacterial diversity represented in the result section through cladograms obtained with Paup* (Swofford 1998). A neighbour-joining analysis was performed using Jukes and Cantor distance. The 116 sequences obtained in this study have been deposited in the GenBank database under accession numbers JX022620 to JX022733.

For direct DAPI counts, the five samples were compared with a Kruskal–Wallis test. Significant differences were determined by the Behrens–Fisher test (Statistica 7.0). For FISH data, a nonparametric Mann–Whitney U test was performed ($\alpha = 0.05$) in order to compare bacterial communities in the sediment and the faeces. As the DNA cloning of only two individuals was used for each season, the seasonal effect versus individual effect was tested with the Jaccard similarity coefficient that is commonly used for comparing the similarity and diversity of sample sets. JSCs were calculated to compare the bacterial diversity (1) between individuals in the rainy season, (2) between individuals in the dry season and (3) between seasons. If the JSC was lower in the third test than in the two first, a seasonal effect could be suspected. A principal component analysis (PCA) showing the bacterial variability that occurred between seawater, substrate and gut segments 1–3 of the digestive tube was performed in Statistica 7.0. For this analysis, a total of 116 clones and isolates (see Table 1) were taken into account. Each variable was a bacterial species, and for each species, the number of retrieved clones was used (this number varies between 0 and 10).

Results

The average number of bacteria per gram (dw) of sediment is shown in Fig. 1 for the five sample locations. The bacterial number significantly increased (×1.5) in the 1st segment of the gut compared to sediment in the substrate ($p < 0.05$). Values reached 11.5×10^9 bacteria per gram in the foregut. The number of bacteria then significantly decreased in the midgut ($p < 0.05$) and stayed stable ($p > 0.05$) in the rest of the digestive tube through the faeces, with values around 4.0×10^9 bacteria per gram.

Results of FISH analyses are shown in Fig. 2. The proportion of bacteria (probe EUB338) in the substrate was not significantly different than the one found in the faeces ($p = 0.146$). The proportion of δ-Proteobacteria (DSS probe) was significantly higher in the substrate ($p < 0.05$) but significantly higher in faeces for the γ-Proteobacteria ($p < 0.05$). There was no significant variation for the Cytophaga–Flexibacter–Bacteroides group (CF probe).

A total of 116 sequences were obtained, among which two putative chimeras that were eliminated (Table 1, details in Figs S1 and S2 in Electronic Supplementary Material). A total of 11 sequences were obtained from seawater, 18 from substrate, 94 from the gut of *H. scabra* and 4 from the faeces. Among the sequences from the digestive tube, 70 were obtained by cloning and 29 from the isolates; 84 sequences were observed in epibenthic individuals and 20 in endobenthic individuals; 63 were retrieved from the dry season, 29 from the rainy season and 1 sequence was observed in the two seasons. The highest number of sequences was obtained from the first segment of the digestive tube (53 phylotypes), then from the second (43 phylotypes) and finally from the third (38 phylotypes).

The observed phylotypes belong to the γ-Proteobacteria, α-Proteobacteria, δ-Proteobacteria, Fusobacteria, Actinobacteria, Cyanobacteria, Bacteroidetes and Firmicutes (Figs S1 and S2). A total of 69 phylotypes were γ-Proteobacteria (Table 1, Fig. S1). This group was the most important of the present study. The genera found were *Coxiella*, *Alteromonas*, *Shigella*, *Vibrio*, *Catenococcus*, *Acinetobacter*, *Halieas*, *Pseudomonas* and *Marinobacterium*. A total of 53 phylotypes grouped with 17 species of *Vibrio*: *V. azureus*, *V. alginolyticus*, *V. fischeri*, *V. harveyi*, *V. natriegens*, *V. proteolyticus*, *V. parahaemolyticus*, *V. campbellii*, *V. rotiferianus*, *V. owensii*, *V. rumoiensis*,

Table 1 Bacterial diversity observed in the sediments transiting through the digestive tract of *H. scabra*. *H. scabra*'s phylotypes are those revealed in the three segments of the gut and faeces of individuals with cloning and culturing methods

Phylotype	Accession number	Closest strain	SL	Taxonomic group	C	M	Se
Isolate *H. scabra* 1	JX022727	*Propionigenium maris*	474	Fusobacteria; Fusobacteriales	JF, A (0,0,1), AF	Culture	Dry
Isolate *H. scabra* 2	JX022733	Fusobacteria	435	Fusobacteria	AF	Culture	Dry
Clone *H. scabra* 3	JX022632	*Elphidium williamsoni*	387	Cyanobacteria	S	Cloning	Dry
Clone *H. scabra* 4	JX022669	*Cyanobacterium* spp.	892	Cyanobacteria	Ep (1,1,0)	Cloning	Dry
Clone *H. scabra* 5	JX022630	α proteobacterium 1	404	Alphaproteobacteria	S	Cloning	Dry
Clone *H. scabra* 6	JX022642	*Balneola alkaliphila*	783	Bacteroidetes; Sphingobacteriales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 7	JX022658	Bacterium spp. 1	936	Bacteroidetes; Sphingobacteriia	Ep (0,1,0)	Cloning	Rainy
Clone *H. scabra* 8	JX022631	*Flavobacterium* sp.	406	Bacteroidetes; Flavobacteriales	S	Cloning	Rainy
Clone *H. scabra* 9	JX022639	*Winogradskyella* sp.	767	Bacteroidetes; Flavobacteriales	Ep (1,0,0)	Cloning	Dry
Clone *H. scabra* 10	JX022664	*Gaetbulibacter saemankumensis*	892	Bacteroidetes; Flavobacteriales	Ep (0,1,0)	Cloning	Rainy
Clone *H. scabra* 11	JX022624	*Sphingobacterium* spp.	409	Bacteroidetes; Flavobacteriia	Ep (0,1,0)	Cloning	Dry
Clone *H. scabra* 12	JX022647	Bacterium spp. 2	855	Bacteroidetes; Flavobacteriia	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 13	JX022699	*Actinobacterium* sp.	845	Actinobacteria	Ep (1,0,0)	Cloning	Dry
Clone *H. scabra* 14	JX022692	*Kocuria palustris*	844	Actinobacteria; Actinomycetales	Ep (1,0,0)	Cloning	Dry
Clone *H. scabra* 15	JX022695	*Microbacterium foliorum*	887	Actinobacteria; Actinomycetales	Ep (1,0,0)	Cloning	Dry
Clone *H. scabra* 16	JX022696	*Solibacillus silvestris*	827	Firmicutes; Bacillales	Ep (1,0,0)	Cloning	Dry
Isolate *H. scabra* 17	JX022721	*Bacillus cibi*	493	Firmicutes; Bacillales	S	Culture	Dry
Clone *H. scabra* 18	JX022626	α proteobacterium 2	386	Alphaproteobacteria	S	Cloning	Dry
Clone *H. scabra* 19	JX022650	*Amaricoccus* sp.	858	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 20	JX022700	α proteobacterium 3	841	Alphaproteobacteria	Ep (1,0,0)	Cloning	Dry
Clone *H. scabra* 21	JX022636	Phyllobacteriaceae	837	Alphaproteobacteria; Rhodobacterales	Ep (1,1,0)	Cloning	Rainy
Clone *H. scabra* 22	JX022646	*Rhodovulum* sp.	760	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 23	JX022654	*Roseobacter* sp.1	863	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 24	JX022689	*Roseobacter* sp. 2	854	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Dry
Clone *H. scabra* 25	JX022652	*Roseobacter* sp. 3	856	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 26	JX022678	*Roseobacter* sp. 4	856	Alphaproteobacteria; Rhodobacterales	Ep (0,0,1)	Cloning	Dry

Table 1 continued

Phylotype	Accession number	Closest strain	SL	Taxonomic group	C	M	Se
Clone *H. scabra* 27	JX022656	*Roseobacter* sp.5	863	Alphaproteobacteria; Rhodobacterales	Ep (0,1,0)	Cloning	Rainy
Clone *H. scabra* 28	JX022627	*Roseobacte*r sp.6	408	Alphaproteobacteria; Rhodobacterales	SW, S	Cloning	Dry
Clone *H. scabra* 29	JX022629	*Roseobacter* sp. 7	382	Alphaproteobacteria; Rhodobacterales	S	Cloning	Dry
Clone *H. scabra* 30	JX022643	*Phaeobacter daeponensis*	831	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 31	JX022655	*Phaeobacter caeruleus*	856	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 32	JX022653	*Tropicibacter* sp.	860	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 33	JX022637	*Ruegeria* sp. 1	786	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 34	JX022638	*Ruegeria* sp.2	795	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 35	JX022651	*Thalassobius gelatinovorus*	857	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 36	JX022677	*Nautella italica*	857	Alphaproteobacteria; Rhodobacterales	Ep (1,0,1)	Cloning	Dry
Clone *H. scabra* 37	JX022660	*Pelagibaca bermudensis*	859	Alphaproteobacteria; Rhodobacterales	Ep (0,1,0)	Cloning	Rainy
Clone *H. scabra* 38	JX022693	*Pelagibaca* sp. 1	834	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Dry
Clone *H. scabra* 39	JX022649	*Pelagibaca* sp 2	855	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 40	JX022641	*Donghicola eburneus*	730	Alphaproteobacteria; Rhodobacterales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 41	JX022644	*Oceanobacterium insulare*	844	Alphaproteobacteria; Rhodobacterales	Ep (1,1,0)	Cloning	Rainy
Clone *H. scabra* 42	JX022640	*Shimia marina*	791	Alphaproteobacteria; Rhodobacterales	Ep (1,0,1)	Cloning	Dry Rainy
Clone *H. scabra* 43	JX022684	*Shimia* sp.	725	Alphaproteobacteria; Rhodobacterales	Ep (0,1,0)	Cloning	Dry
Clone *H. scabra* 44	JX022663	δ proteobacterium	866	Deltaproteobacteria	Ep (0,1,0)	Cloning	Rainy
Clone *H. scabra* 45	JX022645	γ proteobacterium	882	Gammaproteobacteria	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 46	JX022628	*Litoricola marina*	407	Alphaproteobacteria	S	Cloning	Dry
Clone *H. scabra* 47	JX022671	*Coxiella* sp.	881	Gammaproteobacteria; Legionellales	Ep (0,1,0)	Cloning	Dry
Clone *H. scabra* 48	JX022679	Bacterium spp. 3	889	Gammaproteobacteria	Ep (0,0,1)	Cloning	Dry
Clone *H. scabra* 49	JX022635	*Shigella* sp	396	Gammaproteobacteria; Enterobacteriales	S	Cloning	Dry
Isolate *H. scabra* 50	JX022719	*Vibrio azureus* st1	496	Gammaproteobacteria; Vibrionales	Ep (0,0,1)	Culture	Dry
Clone *H. scabra* 51	JX022690	*Vibrio azureus* st2	836	Gammaproteobacteria; Vibrionales	Ep (1,1,0)	Cloning	Dry
Clone *H. scabra* 52	JX022680	*Vibrio azureus* st3	907	Gammaproteobacteria; Vibrionales	SW, Ep (0,0,1)	Cloning Culture	Dry

Table 1 continued

Phylotype	Accession number	Closest strain	SL	Taxonomic group	C	M	Se
Isolate *H. scabra* 53	JX022728	*V. alginolyticus* st1	434	Gammaproteobacteria; Vibrionales	A (0,1,0)	Culture	Dry
Isolate *H. scabra* 54	JX022729	*V. alginolyticus* st2	478	Gammaproteobacteria; Vibrionales	A (1,1,0)	Culture	Dry
Isolate *H. scabra* 55	JX022714	*V. alginolyticus* st3	526	Gammaproteobacteria; Vibrionales	S, Ep (0,0,1), A (1,0,1)	Culture	Dry
Isolate *H. scabra* 56	JX022708	*V. alginolyticus* st4	516	Gammaproteobacteria; Vibrionales	SW,S	Culture	Dry
Isolate *H. scabra* 57	JX022709	*V. alginolyticus* st5	516	Gammaproteobacteria; Vibrionales	S, Ep (1,1,1), J (1,0,1), A (0,0,1)	Culture	Dry
Clone *H. scabra* 58	JX022675	*V. alginolyticus* st6	817	Gammaproteobacteria; Vibrionales	S, Ep (1,0,0)	Cloning Culture	Dry
Clone *H. scabra* 59	JX022691	*V. alginolyticus* st7	907	Gammaproteobacteria; Vibrionales	Ep (0,0,1)	Cloning	Dry
Isolate *H. scabra* 60	JX022720	*V. alginolyticus* st8	516	Gammaproteobacteria; Vibrionales	Ep (0,1,0), A (0,0,1)	Culture	Dry
Isolate *H. scabra* 61	JX022731	*V. alginolyticus* st9	517	Gammaproteobacteria; Vibrionales	Ep (1,1,0), A (0,0,1)	Culture	Dry
Clone *H. scabra* 62	JX022681	*V. alginolyticus* st10	907	Gammaproteobacteria; Vibrionales	Ep (0,0,1)	Cloning	Dry
Clone *H. scabra* 63	JX022668	*V. alginolyticus* st11	908	Gammaproteobacteria; Vibrionales	Ep (0,1,0)	Cloning	Dry
Isolate *H. scabra* 64	JX022715	*V. alginolyticus* st12	526	Gammaproteobacteria; Vibrionales	Ep (1,0,0)	Culture	Dry
Clone *H. scabra* 65	JX022683	*V. alginolyticus* st13	907	Gammaproteobacteria; Vibrionales	Ep (1,1,1)	Cloning Culture	Dry
Clone *H. scabra* 66	JX022685	*V. alginolyticus* st14	878	Gammaproteobacteria; Vibrionales	Ep (0,1,0)	Cloning	Dry
Clone *H. scabra* 67	JX022694	*V. alginolyticus* st15	874	Gammaproteobacteria; Vibrionales	Ep (1,0,0)	Cloning	Dry
Isolate *H. scabra* 69	JX022725	*Vibrio fischeri*	526	Gammaproteobacteria; Vibrionales	J (0,0,1)	Culture	Dry
Isolate *H. scabra* 70	JX022730	*V. harveyi* st1	512	Gammaproteobacteria; Vibrionales	A (1,0,0)	Culture	Dry
Isolate *H. scabra* 71	JX022732	*V. harveyi* st2	504	Gammaproteobacteria; Vibrionales	A (0,0,1)	Culture	Dry
Clone *H. scabra* 72	JX022697	*V. harveyi* st3	879	Gammaproteobacteria; Vibrionales	S, Ep (1,0,0) J (1,1,0), JF, A (1,1,1)	Cloning Culture	Dry
Clone *H. scabra* 73	JX022625	*V. harveyi* st4	410	Gammaproteobacteria; Vibrionales	S	Cloning	Dry
Clone *H. scabra* 74	JX022633	*V. harveyi* st5	368	Gammaproteobacteria; Vibrionales	SW	Cloning	Dry
Clone *H. scabra* 75	JX022673	*Vibrio natriegens* st1	906	Gammaproteobacteria; Vibrionales	Ep (0,0,1)	Cloning	Dry
Clone *H. scabra* 76	JX022667	*Vibrio natriegens* st2	887	Gammaproteobacteria; Vibrionales	Ep (1,1,1), A (0,0,1)	Cloning Culture	Dry
Clone *H. scabra* 77	JX022686	*Vibrio natriegens* st3	908	Gammaproteobacteria; Vibrionales	Ep (1,1,1)	Cloning	Dry
Isolate *H. scabra* 78	JX022713	*Vibrio natriegens* st4	516	Gammaproteobacteria; Vibrionales	Ep (1,0,0)	Culture	Dry

Table 1 continued

Phylotype	Accession number	Closest strain	SL	Taxonomic group	C	M	Se
Clone *H. scabra* 79	JX022666	*Vibrio natriegens* st5	908	Gammaproteobacteria; Vibrionales	Ep (1,1,1)	Cloning	Dry
Isolate *H. scabra* 80	JX022710	*V. proteolyticus*	526	Gammaproteobacteria; Vibrionales	Ep (1,0,0), J (1,0,1), A (0,0,1)	Culture	Dry
Clone *H. scabra* 81	JX022703	*Vibrio parahaemolyticus* st1	874	Gammaproteobacteria; Vibrionales	Ep (1,0,1)	Cloning	Dry
Clone *H. scabra* 82	JX022665	*Vibrio parahaemolyticus* st2	906	Gammaproteobacteria; Vibrionales	Ep (0,1,0)	Cloning	Dry
Isolate *H. scabra* 83	JX022712	*Vibrio parahaemolyticus* st3	516	Gammaproteobacteria; Vibrionales	Ep (0,0,1)	Culture	Dry
Clone *H. scabra* 84	JX022672	*Vibrio parahaemolyticus* st4	907	Gammaproteobacteria; Vibrionales	SW Ep (1,1,1)	Cloning Culture	Dry
Clone *H. scabra* 85	JX022674	*Vibrio parahaemolyticus* st5	907	Gammaproteobacteria; Vibrionales	Ep (0,0,1)	Cloning	Dry
Clone *H. scabra* 86	JX022682	*Vibrio campbellii* st1	907	Gammaproteobacteria; Vibrionales	Ep (1,1,1)	Cloning	Dry
Isolate *H. scabra* 87	JX022711	*Vibrio campbellii* st2	516	Gammaproteobacteria; Vibrionales	J (1,1,1), A (0,1,1), AF	Culture	Dry
Isolate *H. scabra* 88	JX022723	*Vibrio campbellii* st3	526	Gammaproteobacteria; Vibrionales	A (0,1,1)	Culture	Dry
Isolate *H. scabra* 89	JX022724	*Vibrio owensii*	526	Gammaproteobacteria; Vibrionales	J (0,1,1)	Culture	Dry
Isolate *H. scabra* 90	JX022706	*V. fortis* st1	526	Gammaproteobacteria; Vibrionales	SW, J (1,1,1)	Culture	Dry
Isolate *H. scabra* 91	JX022717	*V. fortis* st2	458	Gammaproteobacteria; Vibrionales	Ep (0,1,1), J (0,1,1)	Culture	Dry
Isolate *H. scabra* 92	JX022726	*V. fortis* st3	453	Gammaproteobacteria; Vibrionales	J (0,0,1)	Culture	Dry
Isolate *H. scabra* 93	JX022716	*V. fortis* st4	526	Gammaproteobacteria; Vibrionales	Ep (1,1,1)	Culture	Dry
Clone *H. scabra* 94	JX022705	*Vibrio rotiferianus* st1	829	Gammaproteobacteria; Vibrionales	Ep (0,0,1)	Cloning	Dry
Clone *H. scabra* 96	JX022687	*Vibrio casei*	905	Gammaproteobacteria; Vibrionales	Ep (0,1,0)	Cloning	Dry
Clone *H. scabra* 97	JX022688	*Vibrio rumoiensis*	797	Gammaproteobacteria; Vibrionales	Ep (0,1,0)	Cloning	Dry
Clone *H. scabra* 98	JX022698	*Vibrio hepatarius*	880	Gammaproteobacteria; Vibrionales	Ep (1,0,0)	Cloning	Dry
Isolate *H. scabra* 99	JX022707	*Vibrio brasiliensis*	526	Gammaproteobacteria; Vibrionales	SW	Culture	Dry
Isolate *H. scabra* 100	JX022718	*Vibrio algoinfesta*	436	Gammaproteobacteria; Vibrionales	Ep (0,1,0), J (0,0,1)	Culture	Dry
Isolate *H. scabra* 101	JX022722	*Vibrio neptunius*	516	Gammaproteobacteria; Vibrionales	S	Culture	Dry
Clone *H. scabra* 102	JX022676	*Vibrio* sp. 1	704	Gammaproteobacteria; Vibrionales	Ep (0,1,0)	Cloning	Rainy
Clone *H. scabra* 103	JX022704	*Vibrio* sp. 2	873	Gammaproteobacteria; Vibrionales	Ep (0,1,0)	Cloning	Rainy
Clone *H. scabra* 104	JX022659	*Vibrio* sp.3	906	Gammaproteobacteria; Vibrionales	Ep (0,1,0)	Cloning	Rainy
Clone *H. scabra* 105	JX022670	*Catenococcus thiocycli*	815	Gammaproteobacteria; Vibrionales	Ep (1,1,0)	Cloning	Dry

Table 1 continued

Phylotype	Accession number	Closest strain	SL	Taxonomic group	C	M	Se
Clone *H. scabra* 106	JX022634	*Acinetobacter* sp. 1	367	Gammaproteobacteria; Pseudomonadales	SW	Cloning	Dry
Clone *H. scabra* 107	JX022701	*Acinetobacter* sp. 2	866	Gammaproteobacteria; Pseudomonadales	Ep (0,0,1)	Cloning	Dry
Clone *H. scabra* 108	JX022622	*Acinetobacter* sp. 3	383	Gammaproteobacteria; Pseudomonadales;	SW	Cloning	Dry
Clone *H. scabra* 109	JX022657	*Acinetobacter* sp. 4	949	Gammaproteobacteria; Pseudomonadales	Ep (0,1,0)	Cloning	Rainy
Clone *H. scabra* 110	JX022662	*Acinetobacter* sp. 5	839	Gammaproteobacteria; Pseudomonadales	Ep (0,1,0)	Cloning	Rainy
Clone *H. scabra* 111	JX022702	Acinetobacter sp. 6	870	Gammaproteobacteria; Pseudomonadales	Ep (0,0,1)	Cloning	Dry
Clone *H. scabra* 112	JX022620	*Acinetobacter* sp. 7	391	Gammaproteobacteria; Pseudomonadales;	SW, S	Cloning	Dry
Clone *H. scabra* 113	JX022621	*Pseudomonas putida*	406	Gammaproteobacteria; Pseudomonadales	SW	Cloning	Dry
Clone *H. scabra* 114	JX022623	*Marinobacterium marisflavum*	409	Gammaproteobacteria; Alteromonadales;	S	Cloning	Dry
Clone *H. scabra* 115	JX022648	*Haliea* sp.	857	Gammaproteobacteria; Alteromonadales	Ep (1,0,0)	Cloning	Rainy
Clone *H. scabra* 116	JX022661	*Alteromonas* sp.	892	Gammaproteobacteria; Alteromonadales	Ep (0,1,0)	Cloning	Rainy

Phylotypes retrieved by cloning of seawater and substrates are also indicated. Phylotype names refer to the closest bacterial names obtained during blast search

SL: sequence length; C: compartment; M: method; Se: season; S : sediment, SW : seawater, Ep: epibenthic, JF: endobenthic juvenile, A: endobenthic adult, (1,0,0): foregut,(0,1,0): midgut, (0,0,1): hindgut, (1,1,1): foregut + midgut + hindgut, AF: endobenthic adult faeces

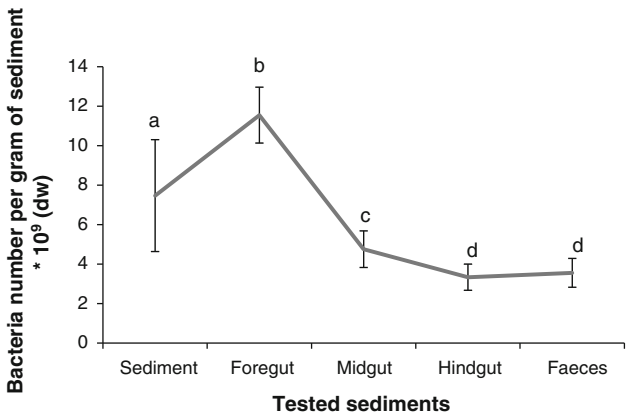

Fig. 1 Bacterial counts as revealed by DAPI staining (mean ± SD; $n = 4$) in the sediment collected in situ, in the foregut, midgut, hindgut and in faeces of *H. scabra*. Values sharing at least one symbol (*a*, *b*, *c*, *d*) did not differ significantly (Behrens–Fisher test; $\alpha = 0.05$)

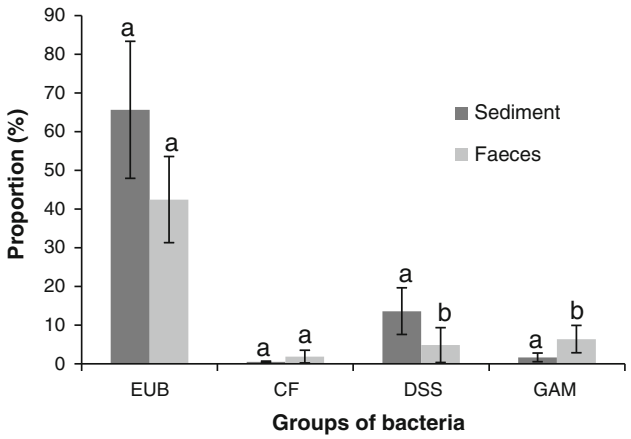

Fig. 2 Bacterial community composition in the substrate (ingested by *H. scabra*) and faeces of *H. scabra* as revealed by FISH analysis (percentages of DAPI counts). *Different symbols* (*a*, *b*) indicate significant differences between substrate and faeces for a bacterial group (*U*-test, $\alpha = 0.05$). EUB: Eubacteria, CF: Cytophaga–Flexibacter–Bacteroides, DSS : δ-Proteobacteria of the *Desulfosarcina-Desulfococcus* group, GAM: γ-Proteobacteria

V. fortis, *V. neptunius*, *V. brasiliensis*, *V. hepatarius*, *V. algoinfesta* and *V. casei* (Fig. S1). The second group comprising an important number of phylotypes belonged to α-Proteobacteria (Fig. S2). The genera found were *Shimia*, *Phaebacter*, *Nautella*, *Roseobacter*, *Donghicola*, *Ruegeria*, *Oceanobacterium*, *Pelagibaca* and *Rhodovulum*. The six other groups comprised between one and seven phylotypes (Fig. S2).

Table 2 Proportion of high ranking taxa identified by cloning from *H. scabra* gut according to the season

Season	Clone number	Proportion of high ranking taxa						
		α-Proteobacteria (%)	γ-Proteobacteria (%)	δ-Proteobacteria (%)	Actinobacteria (%)	Cyanobacteria (%)	Bacteroidetes (%)	Firmicutes (%)
Dry	106	12	79	0	5	3	0	1
Rainy	77	68.5	18.5	2.5	0	0	10.5	0

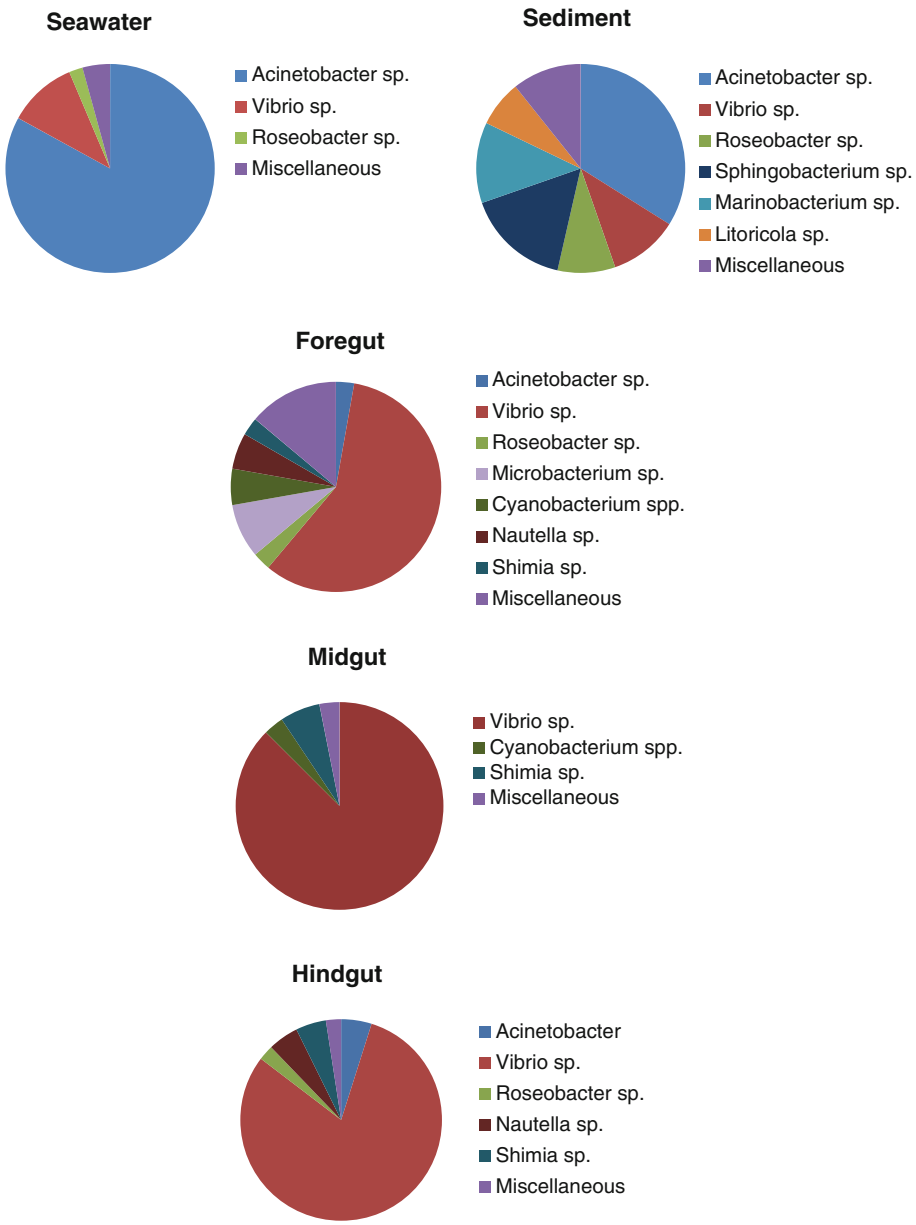

Fig. 3 Bacterial community composition of the seawater, the substrate and the sediments in the three segments of the gut of *H. scabra* as revealed by 16S rRNA sequencing (dry season). Sequence number was 47, 56, 36, 30 and 41 for seawater, sediment and 1st, 2nd and 3rd segments of the digestive tube, respectively

A total of 106 and 77 clones were obtained in the dry (June) and the rainy (January) seasons, respectively (Table 2). The bacterial diversity observed in the sediments of the gut was different in the two seasons. In the dry season, γ-Proteobacteria were the most abundant with 79 % of the sequenced clones, followed by α-Proteobacteria (12 %), Actinobacteria (5 %), Cyanobacteria (3 %) and Firmicutes (1 %). In the rainy season, α-Proteobacteria dominated with 68.5 % of the clones followed by the γ-Proteobacteria (18.5 %), Bacteroidetes (10.5 %) and

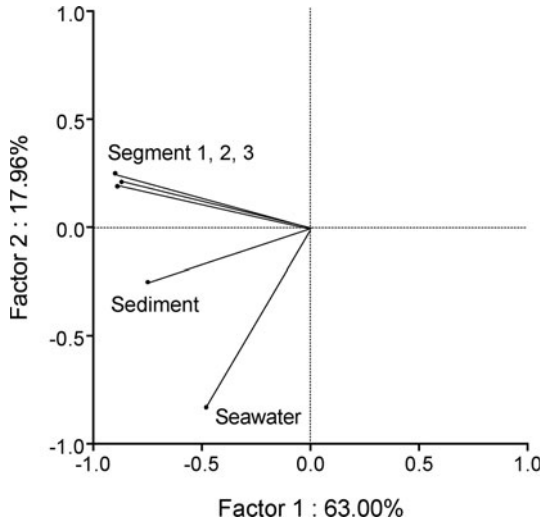

Fig. 4 Principal component analysis (PCA) showing the bacterial variability occurring between seawater, substrate and segment 1–3 of the gut of *H. scabra*

δ-Proteobacteria (2.5 %). The Jaccard similarity coefficient was lower between seasons (JSC = 0.089) than between individuals of the same season (JSC = 0.32 for dry season and JSC = 0.25 for rainy season) suggesting that bacterial communities found in the gut of *H. scabra* were different from one season to another.

The global bacterial community composition of the seawater, of the upper layer of the substrates and of the sediments in the three segments of the digestive tube of *H. scabra* is illustrated on Figs. 3 and 4. For seawater and in situ sediments, most of the clones were identified as *Acinetobacter* (83 % in seawater and 34 % in the substrate). *Vibrio* species were also observed in seawater and substrate but they represented less than 11 % of the clones. *Shigella*, *Pseudomonas*, *Marinobacterium*, *Flavobacterium*, *Sphingobacterium* and three unidentified α-Proteobacteria were also observed in seawater and substrate. The composition of the microbial communities was clearly different in the gut of *H. scabra*, especially for *Acinetobacter* (less present in *H. scabra*'s gut) and *Vibrio* (dominant in *H. scabra)*. *Vibrio* species represented 58, 87 and 80 % of the clones in the 1st, 2nd and 3rd segments, respectively. *Acinetobacter* was found in <5 % of the clones in the 1st and 3rd segments and was absent from the 2nd segment. Similarly, *Roseobacter* was found in less than 3 % of the clones in the 1st and 3rd segments and was not observed in the 2nd segment. Figure 4 illustrates the results of the PCA showing bacterial variability between seawater, substrate and segments 1–3 of the gut. The two first factors of the PCA explain 80.96 % of the variance. Three groups were observed, one clustering the sediments of the three segments together, a second with the substrate and the third with the seawater, the last having a vector almost perpendicular to the one leading to the segments.

Table 3 displays the bacterial isolates obtained from the gut of juveniles and adults of *H. scabra*. A total of 12 phylotypes were obtained and were all identified as *Vibrio* except one isolate of the 3rd digestive segment of the adults that was *Propionigenium maris*. The phylotypes observed in the three stages of *H. scabra* were *V. harveyi*, *V. alginolyticus*, *V. proteolyticus* and *V. campbelli* (but not for all segments). A phylotype close to *V. fortis* was observed in the 3 segments of epibenthic and endobenthic juveniles but was not revealed in adults. Phylotypes close to

Table 3 Comparison of the bacterial isolates observed in the three segments of the gut of *H. scabra*

Bacterial isolates	Epibenthic			Endobenthic					
				Juvenile			Adult		
	Segment 1	Segment 2	Segment 3	Segment 1	Segment 2	Segment 3	Segment 1	Segment 2	Segment 3
Vibrio fortis	1	1	1	3	6	6			
Vibrio harveyi	3	2	2	3	1		5	5	2
Vibrio proteolyticu	1		1	2					1
Vibrio natriegens	1	1	1						1
Vibrio alginolyticus	4	4	3	1			3	2	4
Vibrio campbellii		1		1	1	2		2	1
Vibrio algoinfesta		1			1				
Vibrio parahaemolyticus			1						
Vibrio azureus			1						
Vibrio owensii					1	1			
Vibrio fischeri						1			
Propionigenium maris									1

The bacteria were obtained through 10 bacterial cultures (and sequencing)

V. natriegens, *V. algoinfesta*, *V. parahaemolyticus*, *V. azureus*, *V. owensii* and *V. fischeri* were also observed but were less abundant.

Discussion

Four methods were used in the present study to characterize the bacterial community of sediments transiting through the gut of *H. scabra,* and most of the sequences were identified as *Vibrio* species. It is well known that isolates are dependent on the type of media used and that bacterial cultures do not reflect the real bacterial diversity (Dunbar et al. 1997). The bias in bacterial biodiversity was clear when the retrieved species were compared to those obtained with cloning. Bacterial culture is however a fast, practical method that was useful here to compare bacterial diversity observed in individuals of different ages/size (with different feeding behaviours) and also to compare bacterial strains observed in the various sections of the digestive system (1st, 2nd and 3rd segments). Epibenthic juveniles live on seagrass leaves where they are supposed to feed on the surface microorganisms while endobenthic individuals ingest the surface layer of the substrate during the day (buried during night). Both stages show similar *Vibrio* species in their gut, and we suspected that endobenthic individuals only feed on the superficial layer of the substrate. Cloning is on the other hand a non-culturing approach that is considered as a semi-quantitative method (Acinas et al. 2005). DAPI and FISH counts are true quantitative methods using fluorescent stains or DNA probes (Ravenschlag et al. 2001) that were used here to characterize the number of total bacteria and the number of bacteria from clades of high ranking. However they do not give information on bacterial phylotypes and have to be complemented by the two first methods.

With the use of DAPI counts, we observed a concentration of bacteria around 10^9 g^{-1} in the sediment (dw) of the intertidal zone where *H. scabra* naturally occurs. To date, three previous studies have estimated the number of bacteria in the gut of holothurians. In the first, Ward-Rainey et al. (1996) compared the numbers of colony forming units (cfu) per cm^2 between the foregut and the hindgut in *H. atra*. They observed high cfu in the hindgut which supported the idea of Deming and Colwell (1982) that some bacteria could survive and proliferate in the hindgut. High bacterial numbers in the foregut were also found in the present study. Two other works have suggested that bacterial numbers decrease in the holothurian gut as it is the case in our analysis. Amaro et al. (2012) found a bacterial count of 10^8 cell g^{-1} in sediments of the Nazaré Canyon (NE Atlantic; 3,500 m deep) and a number of 1.26×10^9 cell g^{-1} in the foregut of the deep benthic holothurian *Molpadia musculus*; the number decreased significantly to 2.51×10^8 in the faeces. Taddéi (2006) observed a concentration of 1.5 10^7 bacteria g^{-1} in sediments of shallow waters in La Réunion using a FACScan instrument. This number increased to 3.6×10^7 bacteria $g^{-1}$of sediment (dw) in the foregut of *H. atra* then decreased to 1.91×10^7 in the faeces. The higher number of bacteria in the foregut of holothurians, as observed here and in previous studies, may be explained by bacterial growth in the foregut or by the selection of particles by the holothurian (e.g., selection of particles rich in bacteria).

The digestion of bacteria in the gut of holothurian was estimated to be 80 % for *M. musculus* (Amaro et al. 2012), 53 % for *H. atra* (Taddéi 2006) and 59 % for *H. scabra* (present study). The digestion of bacteria has already been reported by Amon and Herndl (1991) and Moriarty et al. (1985) with efficiencies close to 40 % (Moriarty 1982). Some studies have also demonstrated the digestion of microphytobenthos and more particularly diatom cells (Yingst 1976, Hammond 1983, Uthicke 1999). Our FISH analyses have indicated that faeces contain more γ-Proteobacteria than in situ sediments. This suggests that some γ-Proteobacteria are less affected by digestion than other bacterial groups, a result also supported by our sequence analysis: *Acinetobacter* was the γ-Proteobacterial genus that was the most abundant in the substrate (34 %) and seawater (83 %); on the contrary, *Vibrio* was much more abundant in the gut.

Digestion alone cannot explain the results of the present study. The bacterial community was very different between the substrate and the foregut, with *Vibrio* largely dominating the community of the foregut. It is well known that the role of the foregut is to accumulate food (Féral and Massin 1982). Our results could be explained by the growth and proliferation of *Vibrio* in the foregut or by selection of *Vibrio*-rich sediments. The last hypothesis is supported by a recent analysis (Mezzali and Soualili 2013) demonstrating that the grain size of sediments found in the gut of various Mediterranean holothurians is different from that found in the surrounding substrates. This work supports the view that *H. scabra* is a selective deposit feeder which probably detects and preferentially ingests bacteria-rich sediments. On the other hand, the foregut of *H. scabra* could act as a reservoir where some bacteria such as *Vibrio* proliferate. In his synthesis on the gut microflora in aquatic invertebrates, Harris (1993) already highlighted that resident bacteria can occur in permanent, relatively stable populations that may inhabit pouches of the gut or may be attached to the gut epithelium. Resident bacteria, however, mostly infest the hindgut in aquatic invertebrates (Harris 1993), while resident bacteria in *H. scabra*, if present, would be in the foregut.

Digested bacteria can serve the holothurians in two ways: they can represent a nutrient source and/or their

enzymes may help digestion in the midgut. The first possibility was highlighted in the subtidal *Parastichopus californicus* by Yingst (1976) with ^{14}C labelled bacteria. We also found that *H. scabra* is able to assimilate ^{15}N labelled *Vibrio* (Plotieau 2012). On the other hand, Hatmanti and Purwati (2011) reported that 73.3 % of bacteria in the gut of *H. scabra* produce proteases and 13.3 % produce amylases suggesting that bacterial digestion in holothurian gut could serve to release helpful enzymes.

We observed some phylotypes only in the hindgut possibly because these bacteria could colonize the posterior part of the gut during holothurian breathing. It is also clear that the season greatly influences the microbial communities of the gut. In the dry season, γ-Proteobacteria were very abundant while in the rainy season α-Proteobacteria predominated. *Vibrio* species were very common in the gut of *H. scabra*, a very well-known genus from shallow waters that also includes pathogens of marine animals. *V. harveyi* seems to be common in the gut of *H. scabra*. They are bioluminescent marine bacteria common in tropical marine water. They have often been reported as commensals in the gut microflora of marine animals (Bassler et al. 1997) but they have also been reported as opportunistic pathogens of marine animals, including gorgonian corals, oysters, prawns, lobsters, fishes and holothurians. *V. harveyi,* as well as *V. alginolyticus* another bacteria found in the gut of *H. scabra*, have also been observed in the wounds of the skin ulceration disease (Becker et al. 2004). *V. harveyi* is responsible for luminous vibriosis, a disease that affects commercially farmed penaeid prawns (Liu et al. 1996). *Vibrio parahemolyticus* is a worldwide agent of gastroenteritis and ecological studies demonstrated that it can be isolated from seafood and seawater (McLaughlin et al. 2005, Su and Liu 2007). *V. alginolyticus* can be isolated from the same type of samples as *V. parahemolyticus* and is lethal when injected intraperitoneally in mice (Molitoris et al. 1985). These *Vibrio* are thus potential pathogens for *H. scabra* but at a normal state, they surely contribute to the well growing of individuals.

In conclusion, we demonstrate here that the sediment-associated microbial communities are modified by *H. scabra* during their transit through the gut. This work highlights the role played by *H. scabra,* and presumably by many shallow-water holothurians, in structuring bacterial communities at the sediment–seawater interface.

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