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DIPLOMARBEIT

Titel der Diplomarbeit

„The search for *Candidatus* Endoriftia persephone in skin and tubes of adult *Riftia pachyptila* and *Tevnia jerichonana"*

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Abstract

Since the discovery of the deep-sea hydrothermal vents the symbiosis in Vestimentiferan tubeworms has been studied extensivly, but only a few studies focus on the symbiont transmission. The two closely related tubeworms *Riftia pachyptila* and *Tevnia jerichonana* do not have a mouth or digestive tract. Instead they rely on the symbiont *Canditatus* Endoriftia persephone a chemolithoautotrophic, sulfur-oxidizing gamma proteobacteria, which is harboured in a specialized organ, the trophosome. The symbiont transmission is horizontally through the skin and has to be anew in each host generation during a short time frame in which the juvenile tubeworm larvae settles. In adult tubeworms the symbiont was only found in the trophosome, but a longer time frame in the transmission or a symbiont release from the tubeworm hosts has never been detected so far, like in the squid *Euprymna scolopes,* which release their symbionts daily. To define the transmission time and simultaneous exclude the infection by other bacteria in adults this study focuses on the genetic microbial diversity and differences in the tube and the skin of the co-occurring adult species *Riftia pachyptila* and *Tevnia jerichonana.* In this study, the symbiont 16S rRNA was analyzed by PCR, denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) and fluorescence *in situ* hybridization (FISH) was applied to localize the symbiont in different tissues. The symbiont was found not only in the trophosome, but also in the skin by DGGE and in adult *Riftia* by FISH. The symbiont was also found in the tube by DGGE and T-RFLP of both adult tubeworm species This result suggests either a longer symbiont transmission, which is not only limited to the juvenile stage, or the fact that the symbiont is able to escape from their adult hosts. Surprisingly, another bacterium of the class of Mollicutes was found by DGGE mainly in the skin of *Riftia* but not in the skin or other tissues of *Tevnia*. Mollicutes bacteria are dependent on their hosts and Mollicutes is very well known for their disease-causing role in other organisms for example *Mycoplasma* in humans. Nevertheless, Mollicutes bacteria also occur as a beneficial partner in several symbioses for example *Mesoplasma* in insects. The role of Mollicutes in *Riftia* is going to be analysed here, whereas a beneficial role for *Riftia* cannot be excluded, as Mollicutes could be detected in about 80% of the samples analized.

Zusammenfassung

Seit der Entdeckung der Tiefseehydrothermalquellen wurde die Symbiose in Vestimentifera umfassend untersucht, aber nur ein Bruchteil der Studien befasst sich mit der Symbionten- Transmission. Die beiden eng verwandten Röhrenwürmer *Riftia pachyptila* und *Tevnia jerichonana* haben weder einen Mund, noch einen Verdauungstrakt, sind aber stattdessen von dem Symbionten *Canditatus* Endoriftia persephone abhängig, der ein chemolitho-autotrophes schwefeloxidierendes Gammaproteobakterium ist, welches in einem speziellen Organ, dem Trophosom beherbergt wird. Der Symbiont wird in jeder neuen Wirtsgeneration während eines kurzen Zeitfensters horizontal über die Haut der juvenilen Larve aufgenommen. In ausgewachsenen Röhrenwürmer wurde der Symbiont nur noch im Trophosom gefunden, wobei eine längere Infektionszeitspanne oder ein verlassen des Symbionten aus dem Wirt seither nicht beobachtet werden konnte, wie beispielsweise beim Tintenfisch *Eurpymna scolopes,* welcher seine Symbionten täglich in großen Mengen freisetzt. Um die Infektionszeit durch den Symbionten besser einzuschätzen und gleichzeitig die Infektion anderer Bakterien auszuschließen, wurde in dieser Studie die genetische microbielle Diversität und Unterschiede in der Röhre und in der Haut von ausgewachsenen und gleichzeitig vorkommenden *Riftia pachyptila* und *Tevnia jerichonana* untersucht. In dieser Studie wurde die 16S rRNA des Symbionten mittels PCR, Denaturierende Gradienten-Gelelektrophorese (DGGE), Terminale Restriktionsfragmentlängenpoly- morphismus (T-RFLP) untersucht. Eine genauere Lokalisierung des Symbionten innerhalb der verschiedenen Geweben wurde mittels Fluoreszenz-in-situ-Hybridisierung (FISH) durchgeführt. Der Symbiont wurde nicht nur im Trophosom gefunden, sondern auch in der Haut mittels DGGE und in der Haut ausgewachsener *Riftia* mittels FISH. Desweiteren wurde der Symbiont in beiden Arten der ausgewachsenen Röhrenwüremer in der Röhre mittels DGGE und T-RFLP gefunden. Dieses Ergebnis deutet entweder darauf hin, dass eine längere Symbionten Infektion stattfindet die nicht nur auf das juvenile Stadium beschränkt ist oder darauf, dass der Symbiont in der Lage ist aus dem adulten Wirt zu entkommen. Überraschenderweise wurde ein weiteres Bakterium aus der Klasse der Mollicutes hauptsächlich in der Haut von *Riftia* mittels DGGE gefunden, allerdings wurde es nicht in der Haut oder anderen Gewebeschichten von *Tevnia* gefunden. Mollicutes ist ein von seinen Wirten abhängendes Bakterium und ist für seine krankheitsverursachende Wirkung in anderen Organismen zum Beispiel *Mycoplasma* in Menschen bekannt, allerdings kommt er auch als nutzbringend

für seinen Partner in mehreren Symbiosen vor zum Beispiel *Mesoplasma* in Insekten. Die Rolle, die Mollicutes in *Riftia* übernimmt muss noch untersucht werden jedoch liegt eine für *Riftia* vorteilhafte Wirkung nahe, da Mollicutes in über 80% der untersuchten Proben gefunden wurden.

Introduction

The term symbiosis is used to described the living form of two or more different species that persist together, regardless of the fitness consequences for each partner (de Bary 1879, Mereschkowsky 1905, Lewin 1982, McFall Ngai 2002, Bright & Bulgheresi 2010, Douglas 2010). Three different types of biological symbiotic interactions can be distinguished: commensalistic, mutualistic and parasitic (van Beneden 1875). Commensalistism describes a relationship, where one partner benefits and for the other partner the relationship is considered neutral, whereas mutualistism is described as a form in which both partners benefit from the symbiotic relationship and a parasititic relationship in which one partner benefits while the other one is harmed (Lewin 1982, Leung & Poulin 2008). However the advantage for each of the partners is not always easy to see (see Douglas 2010). Chemosynthetic symbioses between marine invertebrates and bacteria exist for a long time, and such a particular way of symbiotic life is considered to be a required adjustment to the extreme and fluctuating environmental conditions (see Dubilier et al. 2008). *Riftia pachyptila* (Jones, 1981) is a giant deep-sea tubeworm and is one of the fastest-growing invertebrates known, because it has a growth rate of 85 cm/year (Lutz et al. 1994). *Riftia* lives exclusively in the Pacific alongside on the East Pacific Rise attached to cooled lava around the deep-sea hydrothermal vents and at the Galapagos Spreading Center (Cary et al. 1989, Bright & Lallier 2010) together with two co-occuring vestimentiferan tubeworm species *Tevnia jerichonana* (Jones, 1985) and *Oasisia alvinae* (Jones, 1985), which also share the same 16S rRNA phylotype of chemolithoautotrophic symbiont and live both alongside on the East Pacific Rise (Feldman et al. 1997, Di Meo et al. 2000, Bright & Lallier 2010). *Tevnia jerichonana,* which is an early colonizer after volcanic eruptions grows only 30 cm/year (Shank et al. 1998). The vents at the East Pacific Rise are characterized by highly variable temperature from 2°C to 30°C, the pH can be as low as 4.4 and sulphide can be as high as 330 µM (Johnson et al., 1986, Luther et al. 2001, Le Bris et al. 2003, 2006, Haymon et al. 1991). Vestimentiferan tubeworms belong to the

class of Polychaeta, to the family Siboglinidae and do not have a mouth and a digestive tract and they are therefore totaly depend on their nutrients delivering symbiont, which is harboured in a specialised organ called trophosome (Cavanaugh et al. 1981, Felbeck 1981, Felbeck et al. 1985, Cavanaugh, 1985). Nutrients are taken up by the hosts gill-like branchial plume, filled with haemoglobin, which can bind and transport oxygen and sulphide simultanoulsly as well as nitrate to the symbiont in the vascularized trophosome (Arp et al. 1987). In the trophosome the symbiont is located in host cells called bacteriocytes, where the symbiont fixes carbon with two alternative carbon fixation pathways, the Calvin-Benson and the reverse tricarboxylic acid, mainly into malat and succinat (Bosch & Grassé 1984, Markert et al. 2007). These organic acids are nutrients for the host and translocated from the bacteriocytes back through the host body fluids (Arp et al. 1987, Felbeck & Jarchow 1998a).

The symbiont *Cand.* Endoriftita persephone is a gamma proteobacteria with its metagenome sequenced by Robidart et al. (2008), Gardebrecht et al. (2011) and Robidart et al. (2011). A free-living 16S rRNA phylotype of the thiotrophic endosymbiont could be detected in the surrounding hydrothermal vent area with specific primers and visualized with FISH by Harmer et al. (2008). After Endoriftia has infected the settled tubeworm larvae through the skin, the symbiont migrates from the epidermis to the visceral mesoderm. From this tissue the development of the trophosome is initiated by the symbionts (Nussbaumer et al. 2006). After this infection of the juvenile larvae and juveniles the symbiont was never detected in other than the trophosome tissue, but the studies focusing on detecting symbionts around the trophosome and the skin are limited (Nussbaumer et al. 2006). In legumes for example the infection of the symbions happens multiple times during the whole life cycle, but if the legumes die the symbiont can escape their dead host (Kiers et al. 2003). Such an escape from the symbiont Endoriftia persephone in living or dead tubeworms was not detected so far (see Bright & Lallier 2010).

There are two main symbiont transmission modes, which can be distinguished: the vertical transmission in which the symbiont is transmitted from the parents to the offspring like in the earthwurm (*Eisenia foetida)* with its extracellular symbiont *Verminephrobacter eiseniae* or in the vesicomyid clam (*Calyptogena magnifica*), which harbours its intracellular symbiont *Cand.* Ruthia magnifica. The second mode of transmission is the horizontal transmission, where the host gets infected in each generation anew by a symbiont from the environment like the bobtail squid (*Euprymna scolopes*) (see Bright &

Bulgheresi 2010). Additionally, a mixed mode of vertical and horizontal transmission can be found in several symbiotic systems like in the nematode (*Steinernema carpocapsae)* with its symbiont *Xenorhabdus nematophila* (see Bright & Bulgheresi 2010). For a better understanding of symbiosis it is important to understand which transmission mode is used and which impact on the symbiotic system it has. Often vertically transmitted symbionts manifest a reduced genome size. Therefore possible irreversible harmful mutations are accumulated, which can lead to a minor abbility for the symbiont to adapt to environmental changes (Ohta 1973, Moran 1996, Mira & Moaran 2002). In case of a horizontal transmission the symbiont is free-living in the environment and habours a genetically diverse genome wich avoid irreversible mutations and could facilitate an adaptation to environmental changes, but the host has to anew the symbiosis in each generation (see Bright & Bulgheresi 2010). But this new acquisition is mostly limited to a short time frame in which host and symbiont find each other (see Bright & Bulgheresi 2010). To maintain genetic diversity in the genetic pool evolutionary the symbiont has to escape its host and go back into the environment to replenish the environmental pool. Further a release of symbionts from the host into the environment usually occurs in the case of horizontally transmitted symbionts (see Bright & Bulgheresi 2010, Bright & Lallier 2010). The symbiont Endoriftia persephone is transmitted horizontally into the tubeworm host, like the thiotrophic symbiont into the mussel *Bathymodiolus ssp*., but the release of the endosymbiont back into the environment has never been shown from the hosts *Riftia pachyptila* and *Tevnia jerichonana* so far (see Bright & Blugheresi 2010). Because *Riftia pachyptila* and *Tevnia jerichonana* co-occure at the same vent side at the East Pacific Rise in 9° north and harbour the same symbiont 16S rRNA phylotype this study focused on the determination of differences in the distribution of the symbiont *Candidatus* Endoriftia persephone in different tissues between both adult species and between the specimens with a main focus on the skin and tube tissue and whether or not the bacterial distribution is the same. A second main focus in this study was to examine the skin and the tube to determine whether the transmission time frame is longer than just in early juveniles and symbiont infection can happen in adult tubeworms too, or if the symbiont can escape their living hosts. Furthermore, it was examined if infections with other bacteria happen in adults because the skin was referred to as sterile. Because the 16S rRNA phylotype of the symbiont was found all over around the tubeworms and in the water column the symbiont was assumed to be detected at least on the tube wall of the tubeworm (Harmer et al. 2008). To visualize the differences the fingerprint method denaturing gradient gel electrophoresis

(DGGE) was used and for detailed analysis to find differences between the tubes of both adult species a second fingerprint method terminal restriction fragment length polymorphism (T-RFLP) was used. Finally to localize the symbiont in the different tissues fluorescence *in situ* hybridization (FISH) was applied.

Material and Methods

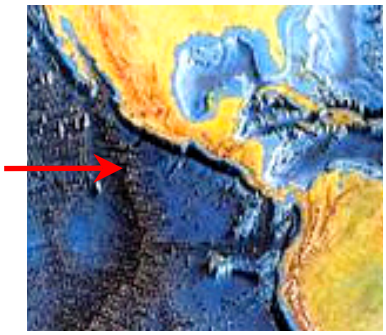

Figure.1: The arrow shows the vent side at 9 50' N EPR (adapted from Heezen & Tharp 1977)

Collection:

Whole specimens of *Riftia pachyptila* and *Tevnia jerichonana* were collected during the cruise ATLANTIS AT 18-12 with the ROV Jason at the East Pacifc Rise (EPR) at the vent sites Tica (9°50.404 N, 104°17.495 W) and P-vent North (9°50.2816 N, 104°17.732 W) in 2500 m depth, with a distance of less than 500 m between both sites in October 2011 (Figure 1, 2B). Both tubeworm species were dissected with a steril set of instruments (scissors and forcepses) on the research vessel immediately after the collection, fixed with 100% ethanol and stored at 4°C. Six *Riftia pachyptila* individuals were separated into three tissue types: trophosome, skin and tube (Figure 2). These subsamples were further separated into top, middle and bottom (Figure 2A,B). Three *Tevnia jerichonana* individuals were separated into two tissue types: the tube and the skin plus trophosome together (Figure 2C).

DNA-Extraction:

Different isolation kits were tested (DNeasy Blood & Tissue Kit (Qiagen), FastDNA spin kit for soil (MP Biomedicals) and UltraClean Soil DNA Isolation Kit (MO BIO Laboratiories)) to determine which would be the most suitable kit for best DNA yield and quality to use for further applications. FastDNA spin kit for soil (MP Biomedicals) revealed the best results according to the manuals instructions. DNA was diluted in 80 µl of DES (DNase/Pyrogen-Free Water) and stored at -20°C for further processing.

16S rRNA amplification:

The 16S rRNA of *Candidatus* Endoriftia persephone was amplified from the extracted DNA with specific primers, RifTO44_F (5'-GGCCTAGATTGACGCTGCGGTA-3') and RifTO445_R (5'TCCTCAGGCTTTTCTTCC-3') (Nussbaumer et al. 2006). PCR was performed with a Mastercycler pro S (vapo. protect) from Eppendorf. One PCR reaction (50 µl) includes 34.3 µl SIGMA Water, 5 µl of 10x buffer with KCl (Fermentas), 5 µl from dNTP Mix (2 mM/ml, Fermentas), 4 µl $MgCl_2$ (25 mM, Fermentas), 0.5 µl specific Primer each (Thermo Scientific), Bovine Serum Albumin (BSA) 0.5 µl (20 mg/ml,Thermo Scienftific), Taq DNA Polymerase 0.2 µl (5 U/µl, Thermo Scientific) and 1 µl of the

extracted DNA. A hot-start touchdown PCR was programmed as follows: initial activation step for 3 min at 95°C, for 35 cycles was the denaturation 30 sec at 95°C, annealing 30 sec at 50°C and the elongation for 45 sec at 72°C, with a final elongation at 72°C for 10 min and the cooled down to 4°C. PCR product was checked by gel electrophoresis with 2 µl size marker (1 kb DNA Ladder, Fermentas), 2 µl 6x Loading Dye (Fermentas) and 2.5 µl template with the PowerPac Basic (Bio Rad) and visualised by SybrGold staining.

Denaturation Gradient Gel Electrophoresis (DGGE):

To examine the microbial diversity and the variations between the different parts, the fingerprinting technique DGGE was used. Specific DGGE primers (Thermo Scientific), 341_F_GC (5'CGCCCGCCGCGCCCCGCGCCCG_CAG-3') and 907_R DGGE (5'- CCGTCAATTCMTTTGAGTTT-3') (Muyzer et al. 1993), were used to amplify the extracted DNA as discribed above for the 16S rRNA. The DGGE gel was prepared with Ingeny phorU-2 with a gradient from 30% (solution A) to 70% (solution B). Solutions A was arranged with 15 ml 0% ureumformamide and 9 ml 80% ureumformamide. Solution B was prepared with 3 ml 0% ureumformamide and 21 ml 80% ureumformamide. Both solutions were mixed and filled up in the compartments of the gradient maker and in a final step 50 µl 20% ammonium persulphate (APS) and 5 µl tetramethylethylenediamin (TEMED) were added and mixed. 250 ml of ureumformamide was mixed before with 2.5 ml 50x Tris-acetate- EDTA buffer (TAE) and with 37.5 ml 40% acrylamide for both concentrates. Additonal the 80% ureumformamide was mixed with 84 g urea and with 80 ml deionised formamide . Both solutions were adjusted to a final volume of 250 ml with water. After polymerisation, solution C was mixed with 7 ml 0% ureumformamide and filled on top of the gel with 70 µl APS and 7 µl TEMED. The polymerised gel was put into 60°C pre-heated 1x TAE buffer bath. A mix of 5 µl amplified DNA, 5 µl loading Dye and 5 µl SIGMA Water was loaded onto the gel. The DGGE was run for 16 h at 100 V with the PowerPac Basic (Bio Rad) and stained with 10 µl SyberGold in 500 ml 1 x TAE buffer for at least 30 min. Subsequently the DNA bands were visualised in the Gel Doc XR^+ Molecular Imager from Bio Rad and cut out for sequencing.

Sequencing:

The cut out bands were transferred into a tube with 50 µl SIGMA Water and stored over night in the freezer at -20°C. PCR was conducted as described above with 1 µl dissolved

band DNA with the primer 341_F (5' CCTACGGGAGGCAGCAG-3') without a GC clamp (Muyzer et al. 1993) and 907_R DGGE (5'- CCGTCAATTCMTTTGAGTTT-3'). Amplified DNA was sequenced with a ABI 3130XL capillary Sanger sequencer from Applied Biosystems. The sequences were compared and blasted (Basic Local Alignment Search Tool) against the NCBI database. Sequence proofreading and final analyses was conducted with the software FinchTV to illustrate the chromatograms of sequences and for sequence searching it were used Geneious.

Embedding/Sectioning:

Fluorescent *in situ* hybridization (FISH) was used to localise the symbionts in the three different body parts. All three tissues and their subdivided parts, stored in 70% ethanol at 4°C, were embedded in LR-White medium resin (London Resin Company Ltd). The samples were dehydrated in a series of ethanol up to 100% ethanol three times for 30 min. The samples were transferred with a sterile pincette and pipette into a 1:1 LR-White to 100% ethanol mixture shaking for 1 h. Afterwards the samples were transferred into pure LR White seven times for 30 min and than infiltrated in pure LR White on a gyratory shaker overnight. The next day LR-White was changed again and samples were infiltrated on a gyratory shaker. The samples were transferred into dried gelatine capsules and closed with the capsules lid for 1 h. A vacuum of 500 mbar was applied to the samples in a desiccator. Samples were polymerised in the desiccator at 45°C for 48 h. The tissues from all *Riftia pachyptila* and *Tevnia jerichonana* samples were cut into 1 µm semi-thin sections with the Microtom UC 7 from Leica and put on gelatine-chromalun ($KCr(SO_4)_2$, 0.5mg/ml) coated slides. The slides were dried in the oven at 40°C overnight.

Fluorescent *in situ* hybridization (FISH):

For FISH all *Riftia pachyptila* skin samples (1540-1542/ 1555-1557), one middle part from the tube (1541 RM), and all skin parts from *Tevnia jerichonana* (1558) were analysed for symbiont and four slides from *Riftia pachyptila* (1541 TM,1541 HM, 1541 HU, 1541 RM) for Mollicutes presence by *in situ* hybridization. The hybridisation buffer (HB) was prepared with 1.09 ml 5 M NaCl, 0.12 ml 1 M Tris/HCl pH 8.2, 6 µl 10% SDS, 2.1 ml 35% formamide and 2.7 ml Milli-Q and a washing buffer (WB) with 700 µl 5 M NaCl, 1 ml Tris/HCl pH 8.2, 50 µl 10% SDS, 0.5 ml 0.5 M ethylenediaminetetraacetic acid (EDTA) and 47.7 ml Milli-Q. Small humid chambers were prepared with kleenex tissues soaked in 2 ml HB and pre-heated at 46°C. Different combinations of probes with the

fluorescence label of FLUOS and Cy3 were combined 1.5 µl of each probe was mixed with 20 µl hybridisation buffer for each section (Table 1). 20 µl of the probe mix was pipetted on each of the fatty-pen surrounded sections on the slides. The slides were incubated in the parafilm sealed humidity chambers at 46° C for 3 h, than washed in 48°C pre-heated WB for 15 min. A NON338 probe (Wallner et al. 1993) was applied as a negative control on one section on every slide investigated. C. Baranyi was sequencing and H. Gruber-Vodicka, PhD. designed the probe Mol753R for this study. Slides were dipped into ice-cold MilliQ and dryed in the dark. Sections were stained with 20 µl with the DNA-binding fluorescent dye 4‘,6-diamidino-2-phenylindole (DAPI, 100 ng/µl) in the dark for 15 min, washed in cold Milli-Q, dryed, mounted with Citifluor and sealed with nail polish. Slides were stored at 4°C until observation on a Zeiss Axio A1 epifluorescence microscope equipped with a AxioCam MRc5 camera from Zeiss.

Terminal restriction fragment length polymorphism (T-RFLP):

To have an detailed digital overview about the tube sample diversity a second fingerprint method T-RFLP were conducted. With this method the microbial distribution pattern from all three tube layers top, middle and bottom of to the two species *Riftia pachyptila* and *Tevnia jerichonana* can be analysed. Fluorescence VIC and FAM labelled primers (Thermo Scientific), 27F-6FAM (FAM-5’-AGA GTT TGA TCC TGG CTC AG-3’) (Moeseneder et al. 1999) and 1492R-VIC (VIC-5′- GGT TAC CTT GTT ACG ACTT-3’) (Fierer & Jackson 2006) were used to amplify the extracted DNA as discribed above for the 16S rRNA. The same PCR cycler was used but with a different programm: initial activation step for 4 min at 95°C, for 30 cycles was the denaturation 1 min at 95°C, annealing 45 sec at 55°C and the elongation for 30 sec at 72°C, with a final elongation at 72°C for 7 min and the cooled down to 4°C. PCR product was checked by gel electrophoresis. Further the PCR product was purified with the Agarose GelExtract MiniKit (5 PRIME). The DNA amount was measured on a NanoDrop 2000 system. Digestion was carried out in 20 µl reaction volume, 100 ng purified DNA, (each from New England Biolabs Ltd.) 0.2 µl restriction enzyme Hha I (20U/ µl), 0.2 µl BSA (20mg/ml), 2 µl of 10x NEB Buffer 4 and Milli-Q was used. The enzyme reaction was incubated for 720 min at 37°C and stopped at 65°C for 20 min. To desalinate the digestion was purified again in a 96-well filter plate using the Sephadex® G-50 purification system. 1,5 µl of each digestion sample was prepared for sequencing with a mastermix: 10 µl HiDi formamide (Biosystems) and 0.3 µl 1200 Liz Standard (Bio-systems). The mix was denatured

on a PCR cycler at 95°C for 3 min and cooled down on ice for 5 min. Amplified DNA was sequenced with a ABI 3130XL capillary Sanger sequencer from Applied Biosystems. The statistical Bray-curtis similarity analysis was conducted with the software Microsoft Excel and Primer 6.

Results

Cand. Endoriftia persephone

The first general validation with the 16S rRNA specific primers for *Cand*. Endoriftia persephone showed the expected results for the trophosome. The symbiont was found in both species. It was present in *Tevnia jerichonana* and *Riftia pachyptila* in the top, middle and bottom subsamples of the trophosome of all 9 specimens examined. *Cand.* Endoriftia persephone was found in all three parts top, middle and bottom in the skin of *Riftia* with PCR and denaturing gradient gel electrophoresis (DGGE) (Table 2). To validate the results from the DGGE, the bands were cut out, sequenced and afterwards analysed and compared with NCBI database. For *Tevnia* the trophosome and skin tissues could not be seperated during the dissection. Therefore it was not possible to discern with PCR and DGGE if the symbiont is really located in the skin or the trophosome.

The endosymbiont was detected with PCR and DGGE in three of six *Riftia* specimens in the top part of the tube and in four out of six *Riftia* specimens in the middle part of the tube. In the bottom part of the tube it was detected in five out of six *Riftia* adults. In *Tevnia*, the symbiont was found once in the top subsample of the tube and in all three subsamples of *Tevnia* in the middle part. It was also found in two of the *Tevnia* specimens in the bottom part of the tube (Table 2).

From all the 16 hybrisizated *Riftia* skin slides the endosymbiont was detected twice in one specimen of *Riftia* (1540 HM), extracellularly located adjacent to the cuticle of the epidermis, extracellularly of the skin (Figure 7), and it was also found in the epithelium intracellularly lining the blood vessel in the skin (Figure 8). Further the tube from *Riftia* (1541 RM) and all the skin parts from *Tevnia* 1558 (THO, THM, THU) was examined with fluorescence *in situ* hybridization (FISH) but the symbiont was not detected.

Mollicutes

The DGGE analysis, from the sequenced bands of the skin and comparison with NCBI database, resulted in many more bands than expected (Figure 5). In the skin of *Riftia* a second microbial bacteria belonging to the group of Mollicutes could be detected in five out of six skin subsamples (Table 2), and in one *Riftia* specimen in the trophosome in the top, middle and bottom part. Mollicutes was found in the top part of the tube and in the bottom part of the tube. In *Tevnia* Mollicutes could not be detected at all in any analysed samples.

I tried to exactly localize Mollicutes with FISH only on four slides with semithin sections all from *Riftia* sample 1541 (TM, HM, HU, RM). A special designed probe Mol753R was used to detect it but I found not a positive signal.

Bacterial diversity

The DGGE analyses of both specimen revealed a wide range of a bacterial community mostly in the tube (Figure 4,6). An overview of the microbial diversity in trophosome, skin and tube of *Riftia* and *Tevnia* is shown in Table 3. Beside of Endoriftia and Mollicutes a variety of uncultured bacteroidetes could be detected in the tube, which belong to the CFB group (Table 3 & Figure 6). In summary there are differences in the top, middle and bottom parts within all three tissue layers (trophosome, skin, tube) between the species and within the specimens but the microbial differsity are in the tube the highest, in the skin the bacterial diversity was almost so low like in the trophosome which Mollicutes and Endorifitia was detected (Tabel 2, 3, Figure 5, 6). In *Tevnia* Mollicutes could not be detected at all in any analysed samples (Tabel 2).

T-RFLP

The comparison of all terminal restriction fragment length polymorphism (T-RFLP) the statistical Bray-curtis similarity analysis with the software Microsoft Excel and Primer 6 shows the bacterial community associated with the tubes in all three parts (middle M, top O, and bottom U) between both species, *Riftia* and *Tevnia* and from the P-vent North location (Figure 3). In the MDS plot the top and the youngest parts of the tube of *Riftia* clearly build a cluster and confront the group which are the middle and the bottom the oldest parts of the tube from *Riftia* build a group. The tubes from all parts of the *Tevnia* samples build not any clearly cluster in confront the *Riftia* samples. The top and the youngest parts of the tube from *Tevnia* build a group and the middle parts of the tube from

Tevnia stick almost together but the bottom and the oldest parts of the tube are randomly spread and do not cluster (Figure 3). The T-RFLP results indicate not a strict separation in carrying the symbiont within the both species, and reflect not a obvious distribution in the three tube parts, top middle and bottom.

Discussion

In this study the symbiont was found not only in the trophosome, but also in the skin by DGGE and in adult *Riftia* in the epithelium lining the blood vessel by FISH. The symbiont was also found in the tube in all three subdivided parts of both adult tubeworm species *Riftia* and *Tevnia* by DGGE and T-RFLP. This result suggests either a longer symbiont transmission, which is not only limited to the juvenile stage, or the fact that the symbiont is able to escape from their adult hosts. Surprisingly, another bacterium of the class of Mollicutes was found by DGGE mainly in the skin of *Riftia,* but not in the skin or other tissues of *Tevnia*. Mollicutes bacteria are dependent on their hosts and Mollicutes could be detected in about 80% of the samples analized, which might suggest a benefical role in *Riftia*.

Due to the fact that the symbiont could be detected in the epithelium intracellularly lining the blood vessel in the skin (Figure 8), a contamination with symbionts during the sample preparation can be excluded.

Host-symbiont distribution

Previous studies:

Cand. Endoriftia persephone has never been detected in the tube and in the skin of adult *Riftia* and *Tevnia* before. López-García et al. 2002 conducted their study with two six year old sampled adults of *Riftia* to find the symbiont in the inner and outer part of the tube and a different DNA extraction method was used in their study. Also, they did not use the specific primers instead they used general bacterial primers 21_F and 1492_R and sequenced cloned aplicons with Genome Express. Using these techniques, the 16S rRNA phylotype could not be detected.

In the study of Nussbaumer et al. 2006 larger *Riftia* juveniles were used to examined the tubes and the skin. Previous studies did not combine FISH and sequencing. In this study a

combination of both methods and new technical standard lead to these new findings of the 16S rRNA symbiont phylotype in the skin and the tube.

Lifelong horizontal transmission:

Finding the symbiont in other than the trophosome may indicate a prolonged horizontal transmission other than known from previous studies. These studies revealed that the infection of the host only takes place in the post-settlement larvae until early juveniles stages (Nussbaumer et al. 2006). According to this study, the host might be infected multiple times during its whole lifetime, similar to legumes where symbionts are able to infect the host multiple times (see Gage 2004). Juvenile *Riftias* are infected by less than 20 symbionts (Nussbaumer et al. 2006). The host is dependent on this small amount of symbionts transmitted to find the best performing symbiotic partner. Therefore, a longer infection could mean that more bacteria are taken up into the adult host during the extended transmission, which further could help the host to better adapt to changes in the environment (see Dubilier et al. 2008).

Apoptosis:

Another explanation of finding the symbiont in the skin could be that apoptosis is not completed in every juvenile as described in (Nussbaumer et al. 2006) and the endosymbiont can persist in the skin until the adult stage.

Symbiont escape from host:

The 16S rRNA phylotype of *Cand.* Endoriftia persephone was detected free-living in the water column of deep-sea hydrothermal vents (Harmer et al. 2008). The symbiont is supposed to live heterotrophically ouside its host, while inside the host it uses the autotrophic pathways. Therefore, the symbiont is considered to be mixotroph (Robidart et al. 2008). Endoriftia is an obligate symbiont for *Riftia,* which means the host is totally dependent on its symbiont, while the symbiont itself can survive without its host easily. The results could lead to the assumption that the symbiont can escape its host after infection as it was described for the squid *Euprymna scolopes*, which releases 95% of their symbiont *Vibrio fischeri* in the morning (Schleicher et al. 2011). *Vibrio fischeri* is released

by its host, but Endoriftia might be able to escape its host actively like it was shown for rhizobia in dead legumes (Kiers et al. 2003). The results of finding Endoriftia persephone in the skin and tube could display the way out of the trophosome for the symbiont in adults. If the symbionts are able to escape their host they replenish also the free-living population (see Bright & Bulgheresi 2010), which also emphasizes the horizontal transmission, as an enlarged pool and availability of the free-living population favours horizontal transmission and the genetic diversity in the genetic pool would be ensured on an evolutionary level (Genkai-Kato & Yamamura 1999). On an evolutionary point of view, the genes of the symbiont would be extinguish when the host dies, if the symbiont can not escape its host (Gardebrecht et al. 2011).

Adaptation to escape or enter an adult host:

The genome of Endoriftia persephone contains chitanase genes (López-García et al. 2002), which theoretically enables it to enter the host through the chitinous tube in both directions (Bright & Lallier 2010). The free-living stage of the symbiont also has a gene for flagellin (Millikan et al. 1999, Robidart et al. 2008) and defense strategies, e.g. type 4 secretion system for the confrontation with the hosts immune system (Robidart et al. 2008). These adaptions are elementary hints, but it is not clear whether and in which time during the lifecycle the genes are expressed and fulfill their entire function.

Microbial differences between Riftia and Tevnia

Due to the fact that the samples of *Riftia* and *Tevnia* where taken at the same time on the same vent site in P-vent North at 9°N and the fact of having a 99,3 % symbiont homogeneity a microbial equal distribution of the two species was assumed (Gardebrecht et al. 2011). Contrary, this study showed differences in the microbial diversity of the tube of the two species from the same sampling site (Table 2,3, Figure 3-6).

Diversity in the tube:

Because the tube is exposed to the environment it offers a surface which can be attacked by microbes other than inner tissues and is therefore much more colonized by bacteria as for example the skin (López et al. 2002) (Figure 4-6).
The symbiont *Cand*. Endorfitia persephone was found in the tubes of *Riftia* from the Tica vent site in a very low abundance and the distribution was mainly localized in the bottom

part of the tube in contrast to the tube of *Riftia* from P-vent North where the symbiont was found in all three specimens in equal distribution. This different symbiont distribution could be a result of the different sampling sites, but there are also differences in the tube of *Riftia* and *Tevnia* which where collected at the same site. In *Tevnia* Endoriftia is distributed in all three middle parts of the tube and in two bottom parts of the tube (Table 2). This could be explained due to chemical variations in local environments, which can have a huge effect and influence on the bacterial growth rate as it was described in bathymodiolin mussels (see Dubilier et al 2008). There is not a clear cluster in the tube samples of *Tevnia* in the T-RFLP analyzes (Figure 3), but differences are visualized in the DGGE (Figure 4). The fact that *Riftia* is a foundation species and *Tevnia* is an early colonizer could explain a shift in the microbial optimum curve. It is possible that bacteria in *Tevnia* are in an optimal growth phase and in *Riftia* the bacteria are just in the beginning of growing (Zwietering et al. 1990). *Tevnia jerichonana* grows closer to the hydrothermal outflow, and it is an early colonizer after volcanic eruptions, because it tolerates relatively low oxygen and relatively high sulfide concentration and probably a higher heavy metals level (Nees et al. 2009). In contrast, *Riftia* needs relatively high oxygen and relatively low sulfide concentrations for growing (Mullineaux et al. 2000, Bright & Lallier 2010, Gardebrecht et al. 2011). These different growth strategies could effect the microbial colonization. *Riftia* has a growth rate of 85 cm/year and grows up to 1.5 m in length, the tube wall thickness is 2-3 mm on average. It is in structure cylindrical, virtually straight, and rather flexible and has no basal partitions, in contrast to *Tevnia,* which grows 30 cm/year, the tube wall thickness is 3-8 mm on the top part on average and is in structure conical tapering and at the basal end, ± twisted and harder (Bright & Lallier 2010, Vrijenhoek 2010). The different structure of the tube wall can influence the uptake and also the colonization of bacteria (Nussbaumer et al. 2006). All these aspects in environmental tolerances and different growth strategies could lead to a different bacterial distribution in both species (Table 2, 3, Figure 3-6).

Mollicutes

Not only Endoriftia persephone but also another bacterium of the family Mollicutes could be detected in the skin of *Riftia* but not in *Tevnia*. Mollicutes was identified in almost all *Riftia pachyptila* samples with a sequence similarity of 94,8 % to the Mollicutes found by Duperron et al. (2012) at the deep-sea region around the Vanuatu Islands in the *Leptochiton boucheti* gut epithelium (Duperron et al. 2012). Whether Mollicutes is located intracellular or extracellular is not clarified yet (Duperron et al. 2012). Mollicutes bacteria

in the study of Duperron et al. (2012) have a 84% sequence identity with Spiroplasma spp. which are commonly located in the gut of different insects (see Whitcomb 1981). The relationship between Spiroplasma family member of the class of Mollicutes and their hosts if Mollicutes are pathogen or mutualistic is not clearified yet (Bové & Garnier 1998) (Table 4). An advantage for *Riftia* could be that the presence of Mollicutes reduces the risk of infection by other pathogens (Wang et al. 2004). A second mutualistic bacterium could help the host to adapt to new environmental changes due to its genetical differences to the symbionts, because a second bacterium makes the host bacteria spectrum more diverse (see Dubilier et al. 2008). Mollicutes are eubacteria of gram-positive origin, with a plasmatic membrane lacking a cell wall (lack peptidoglycan) (Trachtenberg 1998, Rivera-Tapia et al. 2002, Barré et al. 2004, Trachtenberg 2005). The genomes of various Mollicutes bacteria have a low GC content of 24-33 % and the range in size from around 580 kb to 1.4 mb. (Rivera-Tapia et al. 2002, Trachtenberg 2005). Represenatives of Mollicutes can occur in multiple morphotypes e.g. filamentous, helicoidal, ramified and spheroid shape (Rivera-Tapia et al. 2002 and Durand et al. 2010). The eubacteria have an „internal, membrane-bound cytoskeleton“ (Trachtenberg 1998). The bacteria are chemotactic and free-moving but lack flagellas (Razin et al. 1998, Trachtenberg 2005). It can be a pathogen for example in humans *Mycoplasma* or a symbiont for example in *Mesoplasma* insects and Mollicutes bacteria are also totally dependent on its host (Wang et al. 2004, Trachtenberg 2005, Duperron et al. 2012). Mollicutes occurs mostly extracellularly and grows on mucosal surfaces (Trachtenberg 2005, Duperron et al. 2012). The taxonomy of the class of Mollicutes contains four orders (Mycoplasmatales, Entomoplasmatales, Acholeplasmatales and Anaeroplasmatales), which live in different habitats and live in different forms of symbiosis (Johansson & Pettersson 2002) (Table 4). Mollicutes can be associated with sea anemones, ascidians, abalone, *Rimicaris exoculata* and also in humans and plants (Razin 1978, Wang et al. 2004, Durand et al. 2010, Duperron et al. 2012).

But why could Mollicutes not be detected in *Tevnia jerichonana*? A possible explanation is that Mollicutes is species-specific and *Tevnia jerichonana* might have a different surface structure or it has different surface sugars, which makes it impossible for Mollicutes to recognize *Tevnia.* The fact of the different tubewall structures in *Riftia* and *Tevnia* can also influence infection of the Mollicutes (see Bright & Bulgheresi 2010, Bright & Lallier 2010).

In this study, Mollicutes was detected by DGGE and sequencing, but could not be localized by FISH in the samples (TM, HM, HU, RM all from 1541) so far. A possible

explaination is that only a few samples have been analized so far or due to a very low hybridisation signal and/or low bacteria density in the samples (Wang et al. 2004, Durand et al. 2010) or because of the very thin bacteria diameter size of around 0,3 µm (Durand et al. 2010). More samples need to be analyzed by FISH to optimize the probe and detect Mollicutes in the different tubeworm tissues.

Conclusion

This study highlighted that Endoriftia is not only restricted to the trophsome, but can also be present in other tissues of the host. Contrary to previous studies the skin is not sterile, as two bacteria, Endoriftia and Mollicutes could be detected inside the skin. This fact does not only provide a variety of insights into the fade of the thiotroph symbiont in this symbiosis, it also indicates the importance of the symbiont transmission in symbiotic systems, but it also generates questions for the new relationship of Mollicutes in *Riftia.* More analysis have to be conducted to create a new picture of the whole symbiosis to prove the quantity and location of the symbiont in the different tissues to clarify if Endoriftia infects its host for the whole lifetime or escapes from its host. This also applies for Mollicutes. More FISH has to be conducted to localize Mollicutes in the specific tissues to improve the understanding of this potential mutualistic or pathogenic relationship.

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Legends

Table 1: List of FISH probes used, with a mix of three EUB probes to catch most eubacteria. All probes target the 16S rRNA and the different fluorescene labels are listed.

probe	specifity	sequence 5'- 3'	target	label	reference
NON338	negative control	ACT CCT ACG GGA GGC AGC	16S rRNA	Cy3	Wallner et al. 1993
EUB 338 I	most eubacteria	GCT GCC TCC CGT AGG AGT	16S rRNA	FLUOS/ Cy3	Daims et al. 1999
EUB 338 II		GCA GCC ACC CGT AGG TGT	16S rRNA		
EUB 338 III		GCT GCC ACC CGT AGG TGT	16S rRNA		Amann et al. 1990
RifTO445	*Riftia/Tevnia/ Oasisia* symbiont	TCCTCAGGCTTTTCTTCC	16S rRNA	FLUOS/ Cy3	Nussbaumer et al. 2006
Mol753R	Mollicutes	TCC TTT CAT GCC TCA ACG	16S rRNA	FLUOS/ Cy3	this study

Table 2: *Cand*. Endoriftia persephone detected by PCR (P) and DGGE (D) in both, *Riftia* and *Tevnia* as well as Mollicutes (M, highlighted in blue) detected by DGGE (D) in *Riftia,* but not in *Tevnia.* The validation was done in all three tissue layers, trophosome (T), skin (H) and tube (R) and their subdivision parts, top (O), middle (M) and bottom (U). The + indicates presence and the - indicates absence.

tissue	TO			TM			TU			HO			HM			HU			RO			RM			RU		
method	P	D	M	P	D	M	P	D	M	P	D	M	P	D	M	P	D	M	P	D	M	P	D	M	P	D	M
1540	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	+	+	+
1541	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-
1542	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-
1555	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
1556	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-
1557	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-
1558	+	+	-	+	+	-	+	+	-			-			-			-	-	-	-	+	+	-	+	+	-
1559	+	+	-	+	+	-	+	+	-			-			-			-	+	+	-	+	+	-	+	+	-
1560	+	+	-	+	+	-	+	+	-			-			-			-	-	-	-	+	+	-	-	-	-

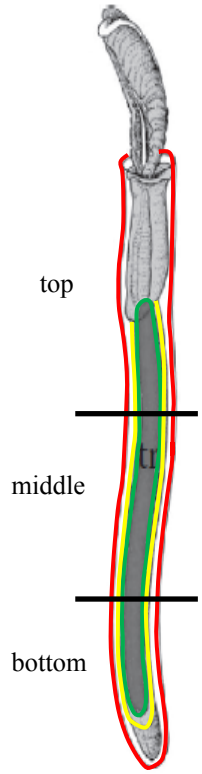
Table 3: Overview of the sequenced microbial diversity of all DGGE bands of *Riftia* and *Tevnia* from the trophosome, skin and tube.

	Riftia	***Tevnia***
trophosome (T)	Endorifitia persephone	Endorifitia persephone
	Uncultured Mollicutes bacterium clone	
skin (H)	Endorifitia persephone	
	Spiroplasma endosymbiont of *Drosophila tenebrosa*	
tube (R)	uncultured Endorifitia persephone	uncultured alpha proteobacterium
	uncultured Bacteroidetes bacterium clone	uncultured CFB group bacterium clone
	uncultured CFB group bacterium clone	CFB group bacterium epibiont of *Rimicaris exoculata*
	Olavius algarvensis spirochete endosymbiont	CFB group bacterium of *Microscilla aggregans catalitica*
	Olavius crassitunicatus spirochaete endosymbiont	*Sulfitobacter pontiacus*
	Olavius loisae endosymbiont	
	uncultured delta proteobacterium clone	
	uncultured epsilon proteobacterium clone	
	uncultured Arcobacter sp.	
	uncultured Cytophagales bacterium clone	
	uncultured Myxococcales bacterium clone	
	Uncultured sedimnet bacterium	
	uncultured Spirochaetes bacterium clone	
	uncultured Verrucomicrobia bacterium	

Table 4: The taxonomy of the class of Mollicutes contains four orders, which occur in different habitats. Their different lifestyle tendency of symbiosis are listed (adapted from Johansson & Pettersson 2002).
* the genus of Cand. Phytoplasma is not clarified because it is still uncultured (Malembic-Maher et al. 2011).

order	**family**	**genus**	**habitat**	**reference**
Mycoplasmatales	Mycoplasmataceae	Mycoplasma Ureaplasma	humans, animals (pathogen)	Rivera-Tapia et al. 2002
Entomoplasmatales	Entomoplasmataceae	Entomoplasma Mesoplasma	insects, plants (mutualsm/pathogen)	Bové & Garnier 1998
	Spiroplasmataceae	Spiroplasma	insects, plants (mutualsm/pathogen)	Gasparich 2010
Acholeplasmatales	Acholeplasmataceae	Acholeplasma	animals, plant surfaces (pathogen)	Rivera-Tapia et al. 2002
		Cand. Phytoplasma*	Insects, plant (pathogen)	Malembic-Maher et al. 2011
Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma Asteroleplasma	bovine and ovine rumen (pathogen)	Rivera-Tapia et al. 2002

A



B

no.	species	tissue layer	parts	abbreviation	location	year
1540	*Riftia*	trophosome	top	TO	Tica	10/2011
			middle	TM		
			bottom	TU		
		skin	top	HO		
			middle	HM		
			bottom	HU		
		tube	top	RO		
			middle	RM		
			bottom	RU		
1541	*Riftia*	s.a.	s.a.	s.a.	Tica	10/2011
1542	*Riftia*	s.a.	s.a.	s.a.	Tica	10/2011
1555	*Riftia*	s.a.	s.a.	s.a.	P-vent north	10/2011
1556	*Riftia*	s.a.	s.a.	s.a.	P-vent north	10/2011
1557	*Riftia*	s.a.	s.a.	s.a.	P-vent north	10/2011
1558	*Tevnia*	trophosome and skin	top	THO	P-vent north	10/2011
			middle	THM		
			bottom	THU		
		tube	top	RO		
			middle	RM		
			bottom	RU		
1559	*Tevnia*	see above	s.a.	s.a.	P-vent north	10/2011
1560	*Tevnia*	s.a.	s.a.	s.a.	P-vent north	10/2011

C

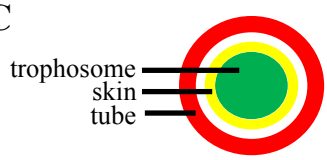


Figure 2: (A) *Riftia pachyptila* illustration with all three parts top, middle and bottom and the subdivided tissue layers, trophosome (green), skin (yellow) and tube (red). (B) Table with all samples of *Riftia pachyptila* and *Tevnia jerichonana* with their sample number (no.), tissue layer: trophosome (green), skin (yellow) and tube (red) and the three subdivided parts: top, middle and bottom. Sampling side and year are displayed. (C) The different subdivision of disected *Tevnia jerichonana* with two parts: trophosome (green) and skin (yellow) stick together and the second part, the tube (red).

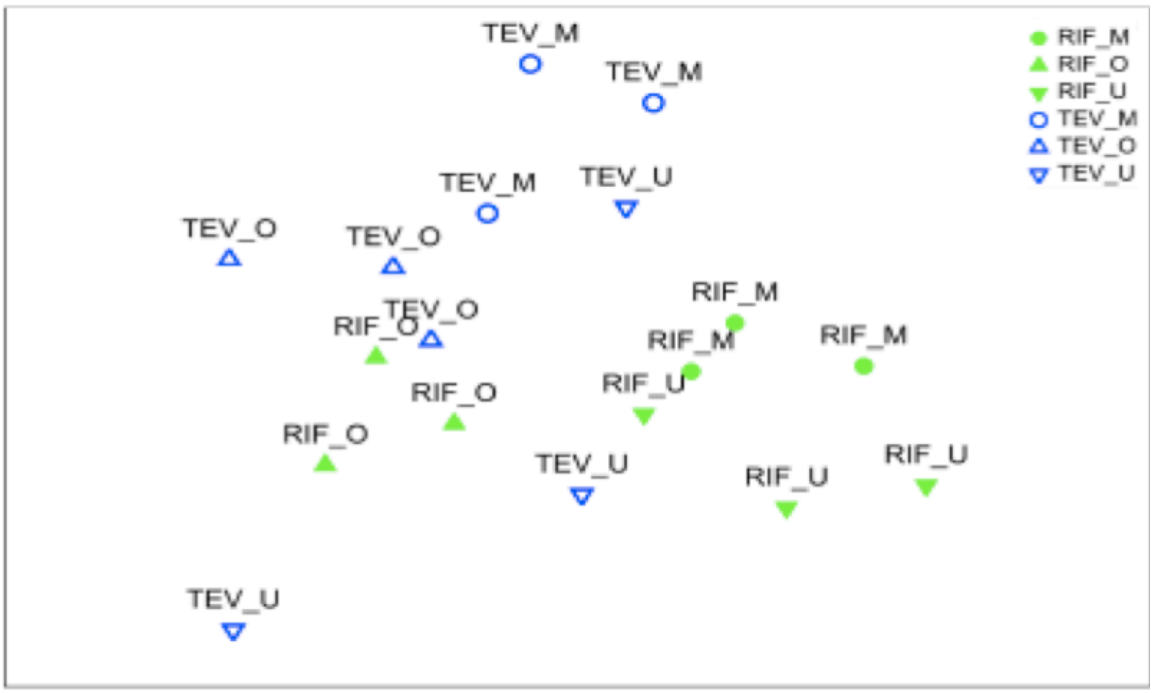


Figure 3: T-RFLP comparison of the tubes of all three subdivided parts middle (M), top (O) and bottom part (U) between both species, *Riftia* (RIF) and *Tevnia* (TEV), from the P-vent North location. The top samples of *Riftia* cluster together as well as the middle and bottom part of the *Riftia* samples, which build a clear cluster. The bottom parts of *Tevnia* do not display a distinct pattern. The middle parts of *Tevnia* stick together and the top parts of the tube form a group, but not a definite cluster.

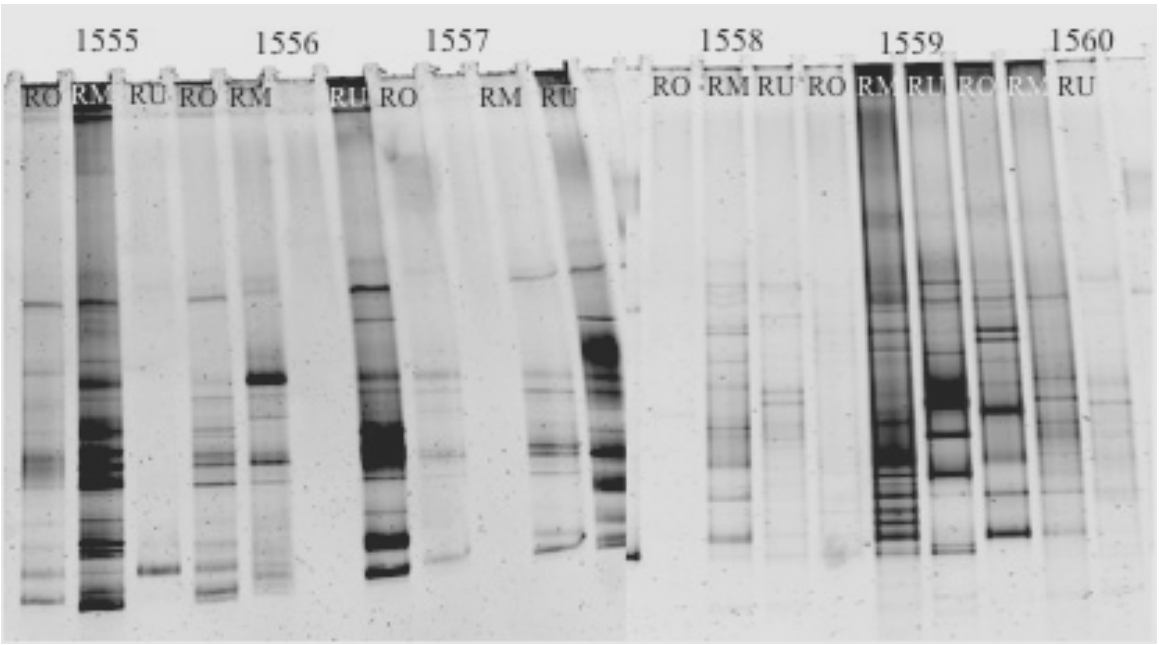


Figure 4: DGGE comparison of the tubes (R) of all three subdivided parts top (O), middle (M) and bottom (U) between both species, *Riftia* (1555-1557) and *Tevnia* (1558-1560), from the P-vent North location. There is a different microbial distribution of both species in the tube.

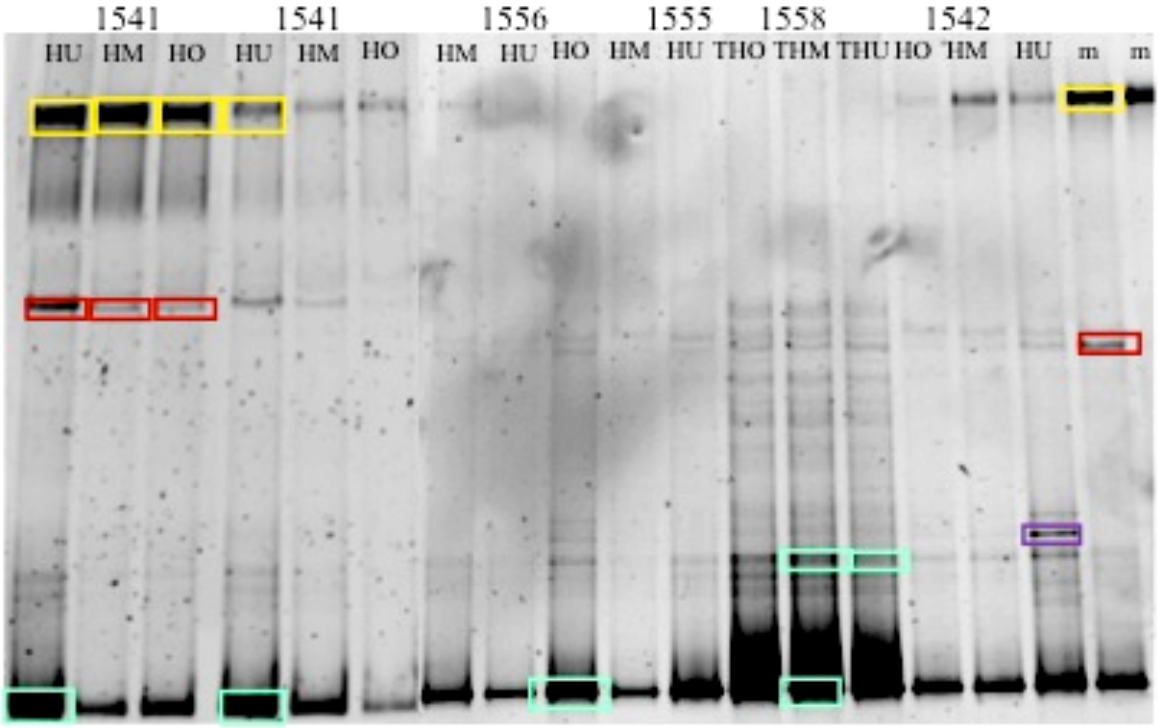


Figure 5: The marked band display the cut out and sequenced DGGE bands from the skin (H) with all three subsamples top (O), middle (M) and bottom (U) compared with a marker (m), which is used as a template and contains all three bands (*Cand.* Endoriftia persephone (green), a mix between Endoriftia and Mollicutes (red) and *Cand.* Mollicutes bacterium).

Cand. Endoriftia persephone — Mix between Endoriftia and Mollicutes

Cand. Mollicutes bacterium — Uncultured bacterioidales bacterium

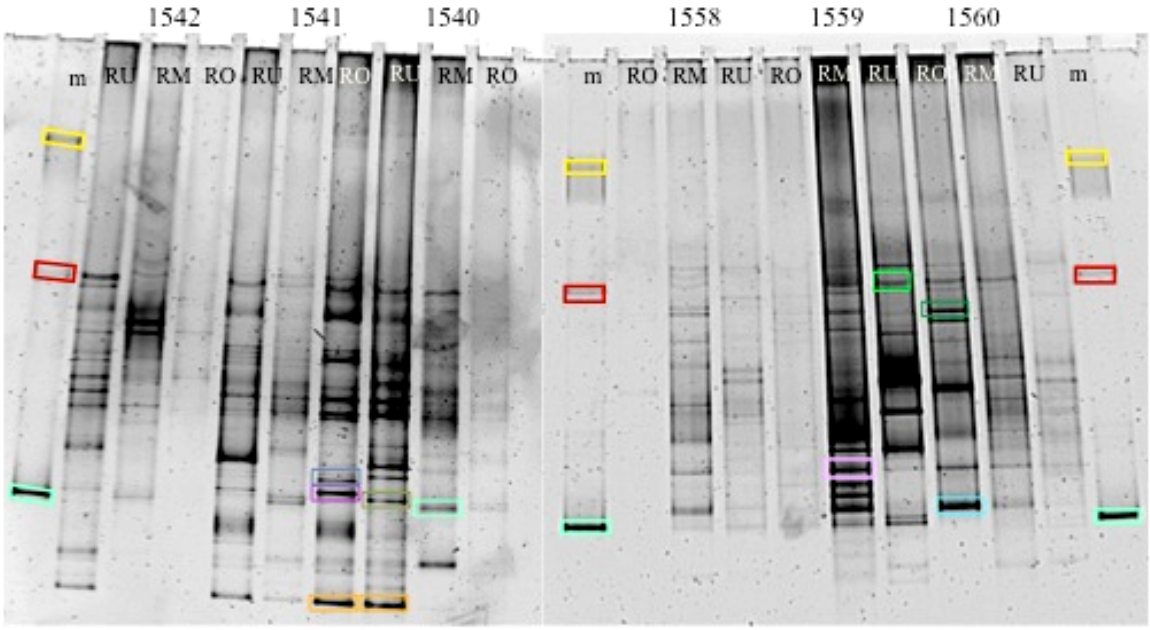


Figure 6: The marked bands display the cut out and sequenced DGGE bands from the tube (R) with all three subsamples top (O), middle (M) and bottom (U) compared with a marker (m), which is used as a template and contains all three bands (*Cand.* Endoriftia persephone (green), Mix between Endoriftia and Mollicutes (red) and *Cand.* Mollicutes bacterium).

- Cand. Mollicutes bacterium
- Mix between Endoriftia and Mollicutes
- Cand. Endoriftia persephone
- Uncultured CFB group bacterium
- Uncultured bacterium
- Uncultured bacterium
- Uncultured CFB group bacterium
- Sulfitobacter pontiacus
- Uncultured CFB group bac. epibiont on *Rimicaris exoculata*
- Uncultured alpha proteobacterium
- Uncultured delta proteobacterium

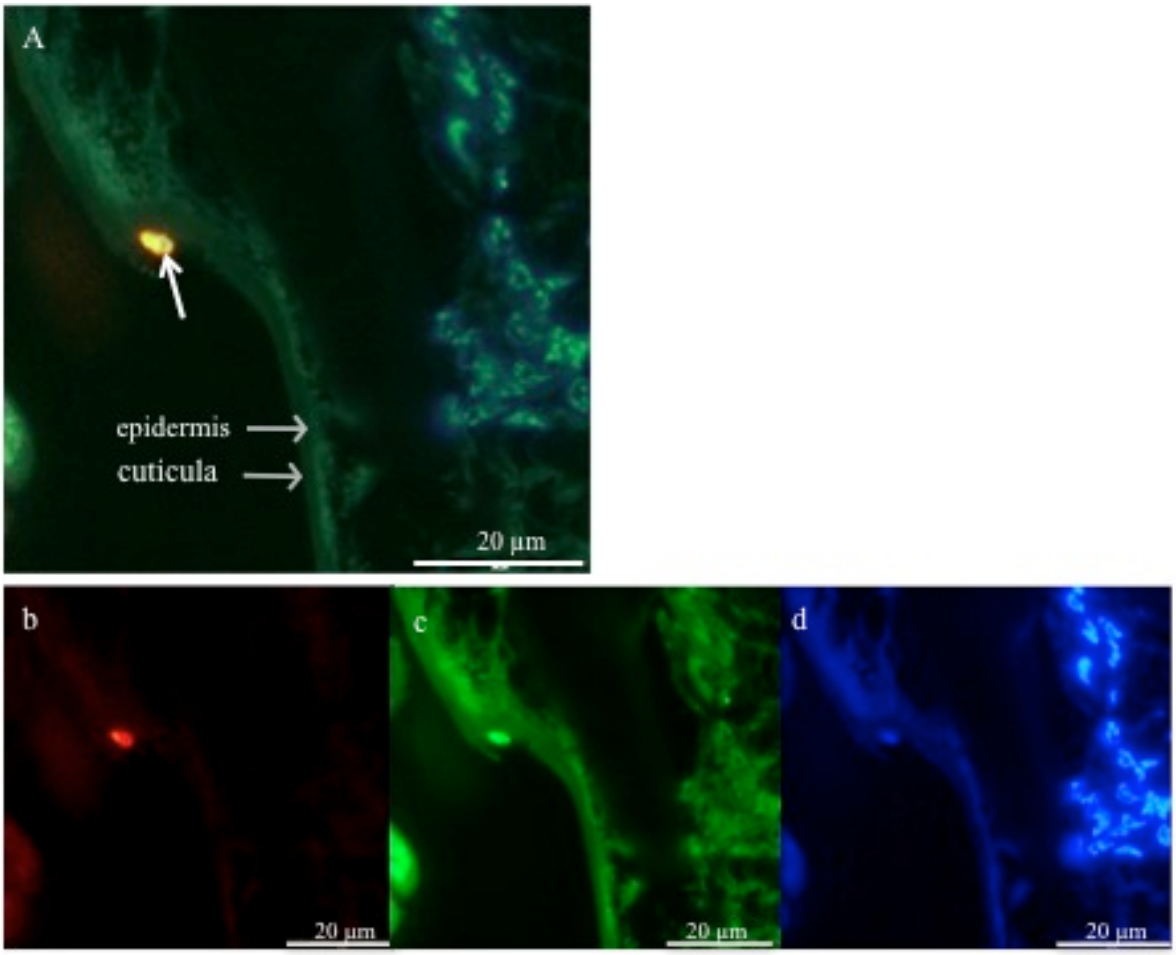


Figure 7: FISH micrograph showing the symbiont *Endoriftia persephone* (arrow) extracellular on the cuticula of the skin in the overlay (A) of all three images from one *Riftia pachyptila* specimen (1541 HM). In detail FISH on semithin sections in three different probes: RifTO 445 (red) (b), Eubacteria EUB-Mix 445 (green) (c), DAPI (blue) (d) are displayed.

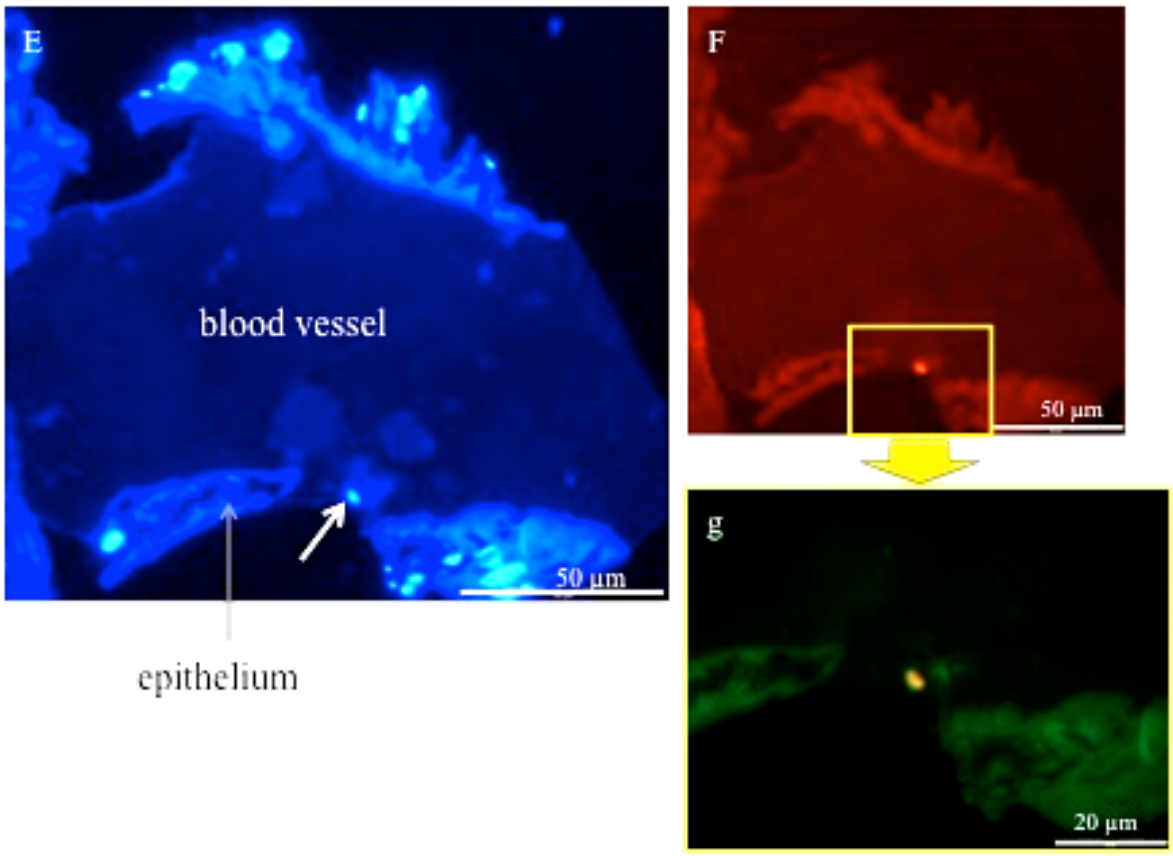


Figure 8: FISH micrograph showing the symbiont Endoriftia persephone (arrow) intracellular in the epithelium lining the blood vessel in the skin of *Riftia pachyptila* (1541 HM) in DAPI (blue) (E) and in RifTO 445 (red) (F). In detail the overlay of two images with symbiont-specific probes (red) and Eubacteria EUB-Mix (green) (g) is magnified.

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POSTER

*Buck, A., *Scharhauser, F., Klose, J. & Bright, M. (2013): Candidatus Endoriftia persephone and Mollicutes in the skin and tube of adult vestimentiferan tubeworms from deep-sea hydrothermal vents. *5th International Symposium on Chemosynthesis-Based Ecosystems* in Victoria, (Canada).
* Diploma thesis with equal contribution to this study